# 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, spinner flasks or Applikon AppliFlex 3L and Xcellerex XDR-10 single-use (SU) stirred bioreactors with an inoculum density of $1.5x10^5$ cells/mL. Cell growth studies were performed for 72-120h. The shake and spinner flasks were kept at 37°C with 5% $CO_2$ and the bioreactors at 37°C, 40% dO and pH 7.2. 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-dimen¬sional (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 AAV2 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 to 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 at 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 Cellevat3d® nanofiber microcarriers formed homogeneous 3D cultures, generating spheroids with an average diameter of 179 µm after 96h **(Figure 1A,B).** The scalability of the microcarrier cultures was demonstrated by successful scale-up from spinner flasks (milliliter to liter scale) to a 2.4L Applikon AppliFlex SU stirred bioreactor and a 10L Xcellerex XDR SU stirred bioreactor, maintaining consistent cell growth curves and achieving cell densities up to $3x10^6$ cells/mL, with viability above 95% throughout the culture **(Figure 1C,D).**

### 3D cell culture scale-up using Cellevat3d® nanofiber microcarriers

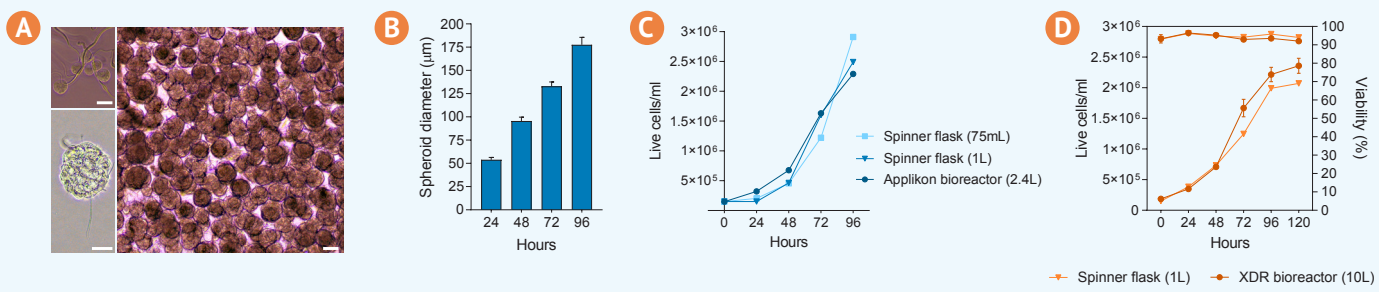



**Figure 1.** Scalability of Cellevat3d® nanofiber microcarrier cell cultures. **A** Bright field microscope images of HEK293T cells adhering to nanofiber microcarriers at inoculum and growing as homogeneous 3D spheroids, shown after 72h in culture. Scale bars 25µm (left top), 50µm (left bottom) and 100µm (right). **B** Nanofiber microcarrier cultures (75mL spinner flask) generate 3D spheroids that gradually increase in size over time reaching an average diameter of 179±8 µm after 96h (n=50 measurements per timepoint). **C** Scale-up from 75mL spinner flask to 1L spinner flask and 2.4L Applikon AppliFlex SU stirred bioreactor resulted in consistent cell growth, reaching yields up to $3x10^6$ cells/mL in 96h. **D** Scale-up from 1L spinner flask to 10L Xcellerex XDR SU stirred bioreactor maintained similar cell growth trends and an average cell viability of 94% throughout the culture (n=2 independent experiments). Data are presented as either mean or mean ± SEM.

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## Viral vector production using Cellevat3d® nanofiber microcarriers

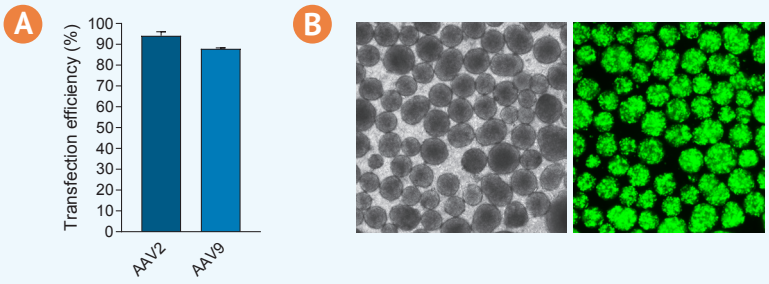



**Figure 2.** Transient transfection of HEK293T cells on Cellevat3d® nanofiber microcarriers. A Plasmid transfection efficiency (%) measured by flow cytometry for AAV2 and AAV9 (n=3-6 biological replicates). B Fluorescence microscope images of ZsGreen-expressing transfected 3D spheroids at 72h post-transfection.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

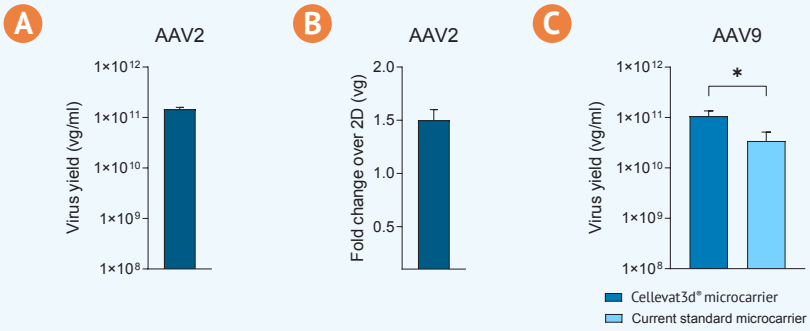



**Figure 3.** Volumetric productivity of AAV vectors using Cellevat3d® nanofiber microcarriers. A AAV2 titer of $1.5x10^{11}$ vg/mL measured by qPCR (n=4 biological replicates) from 20mL culture. B Increased product yield (1.5-fold) over parallel AAV2 production using 2D (culture flasks) with same number of cells. C Benchmarking data showed 3.1-fold significant increase in AAV9 titer when using Cellevat3d® nanofiber microcarriers compared to current standard microcarriers (n=6 biological replicates). Data are presented as mean ± SEM. p* < 0.05, using the Mann–Whitney U test.

## Results II

Transient plasmid transfection of 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)** with higher titers compared to parallel 2D cultures **(Figure 3B).** Recent benchmarking results conducted by UCL in London demonstrated a 3.1-fold significant increase in AAV9 vector titer using 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 scale-up in bioreactors. Our data demonstrated scale-up in a 10L SU stirred bioreactor, as well as high transfection efficacy and volumetric productivity of AAV vectors using Cellevat3d® nanofiber microcarriers. Most importantly, benchmarking results showed 3.1-fold increase of AAV9 vector titer using 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 are available to order on www.cellevate.com.

## References


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