Cellevate

APPLICATION NOTE

More than 10-fold enhanced viral vaccine production titers using the novel Cellevat3d® nanofiber microcarriers

Introduction

Current upstream techniques for adherent viral vaccine production rely on bead-based microcarriers, roller bottles, cell stacks and fixed-bed bioreactors. While these methods are well established and proven across many processes, vaccine production faces tremendous cost pressures, particularly when addressing large populations or within the growing veterinary vaccine sector. Additionally, with novel applications of viral vaccines, such as oncolytic vaccines for cancer treatment, it is critical to manufacture these therapies at a sustainable cost to safely treat large patient populations.

Therefore, developing new virus production methods that deliver higher titers and productivity, reduce process complexity, enhance ease of use and process robustness, and enable rapid process transfer and scalability is essential. Furthermore, as sustainability becomes increasingly important for companies and economies, the use of natural, biodegradable materials offers significant benefits.

To address these challenges, Cellevate has developed Cellevat3d® VAX, a novel nanofiber-based cell growth support specifically optimized for adherent cells such as Vero cells, which are widely used in viral vaccine production. Cellevat3d® VAX nanofiber microcarriers are designed to deliver unmatched infectious virus titers and to enable rapid, straightforward production scale-up based on a sustainable cellulose-derived nanofiber material.

The use of Cellevat3d® nanofibers provides a high surface area and enables controlled formation of three-dimensional (3D) spheroids from anchorage-dependent cells, resulting in dense, viable cell populations ideal for efficient virus propagation and production. Moreover, the nanofiber microcarriers are fully compatible with existing stainless steel and single-use stirred tank bioreactors, facilitating seamless process transfer and scale-up within existing production facilities.

Aim

This application note demonstrates the advantages of the Cellevat3d® nanofiber platform for viral vaccine production by evaluating production strategies for various viral vaccines using Vero and HEK 293T cell cultures. The data were generated by Cellevate AB and Intravacc B.V., a CDMO with extensive expertise in vaccine manufacturing.

Cellevat3d® nanotechnology platform for industrial cell culture

Cellevat3d® VAX is a novel cell growth support specifically optimized for adherent culture of Vero cells and other strongly adherent cell types in stirred tank bioreactors. It is based on the Cellevat3d® nanotechnology platform, designed to enhance production titers in viral vaccine as well as HEK 293 cell-based viral vector systems. The Cellevat3d® nanofiber platform is flexible and can be easily customized to meet the specific requirements of different cell lines.

Advantages of Cellevat3d® nanotechnology

Cellevat3d® nanotechnology	Advantages
Up to 85% reduction in cost of biotherapies production	Unmatched virus titers Reduced waste Reduced water use Faster bioprocessing time Enhanced productivity
3 to 11-fold increase in virus titers compared to current solutions	Improved yields and productivity for viral vectors and viral vaccines
Large surface area for adherent cells	Cell-dense spheroid cultures with high viability
Consistent cell growth across different bioreactor scales	Proven scalability in stirred tank bioreactors
Enzyme-free cell passages	Easy and rapid seed-train expansion
Versatility in raw material, nanofiber size, surface functionalization, scaffold architecture, bioreactor compatibility and application areas	Flexibility to meet customer demands
Ease of use	Depth filtration removal – no change to USP & DSP steps
Based on cellulose: biodegradable, sustainable, and green raw material	Sustainability
ISO 9001 and GMP	High quality

Table 1. Advantages of Cellevat3d® nanotechnology, see also **Ref 1 and 2**.

Cellevat3d® commercial products for industrial cell culture

Product Name	Application	Product Code
Cellevat3d® AAV/LV nanofiber microcarriers	For adeno-associated virus (AAV) and lentiviral (LV) production	200-01 For larger sizes, please contact sales team at sales@cellevate.com
Cellevat3d® VAX nanofiber microcarriers *Commercially available in October 2025*	For viral vaccine production	For pre-ordering, please contact sales team at sales@cellevate.com

For more information, visit www.cellevate.com

NEW! Cellevat3d® VAX nanofiber microcarriers for viral vaccine production



Material and Methods

1. Vero cell growth study using Cellevat3d® VAX nanofiber microcarriers

Cellevat3d® VAX nanofiber microcarriers were resuspended in Dulbecco's phosphate buffered saline (DPBS) without calcium and magnesium to prepare a stock solution of 5 mg/mL and subsequently autoclaved. Vero cells (CCL-81™ cells, ATCC) were seeded at 2×10^5 cells/mL in spinner flask together with sterilized nanofiber microcarriers at a final concentration of 0.1 mg/mL final concentration in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% GlutaMAX™, and 1% sodium pyruvate, reaching a working volume of 80mL. The Vero cells were grown for 72h in spinner flasks placed on a magnetic stirrer at 80 rpm inside a 37°C humidified incubator with 5% CO_2. Viable cell density (VCD) was assessed at 24h, 48h, and 72h using the NucleoCounter® NC-202™. In parallel, 2D cultures were run under the same medium conditions, and cell numbers were measured at 72h to compare growth as fold change over the initial seeding density.

2. Enterovirus A71 production study with Vero cells at different multiplicity of infection using Cellevat3d® VAX nanofiber microcarriers

Vero cells were inoculated at a density of 2×10^5 cells/mL in shake flask together with sterilized nanofiber microcarriers (0.075mg/mL final concentration) in DMEM with 10% FBS, 1% GlutaMAX™, and 1% sodium pyruvate, reaching a working volume of 30mL. The Vero cells were grown in shake flasks placed on an orbital shaker (19 mm) at 90 rpm inside a 37°C humidified incubator with 5% CO_2. After 72h of cell growth, the cells were infected at a density of $0.8\text{x}10^6$ cells/mL with enterovirus A71 (EV71) (VR-1775TM, ATCC) at a multiplicity of infection (MOIs) of 0.01, 0.001 or 0.0001. Viruses produced by the infected cell cultures were sampled daily up to day 10 post-infection. Infectious virus titers were determined using the TCID50 assay (ATCC).

3. Enterovirus (non-disclosed) production benchmarking with HEK 293T cells using Cellevat3d® nanofiber microcarriers and industry standard microcarriers (study performed by Intravacc B.V.)

In a benchmarking study conducted by Intravacc B.V., an independent Contract Development and Manufacturing Organization within the vaccine industry (www.intravacc.nl), Cellevat3d® nanofiber microcarriers were compared to industry standard microcarriers for enterovirus (non-disclosed) production using HEK 293T cells.

Cellevat3d® nanofiber microcarriers were resuspended in PBS to prepare a stock solution of 5 mg/mL, and subsequently autoclaved. Industry standard microcarriers were prepared according to the manufacturer's instructions.

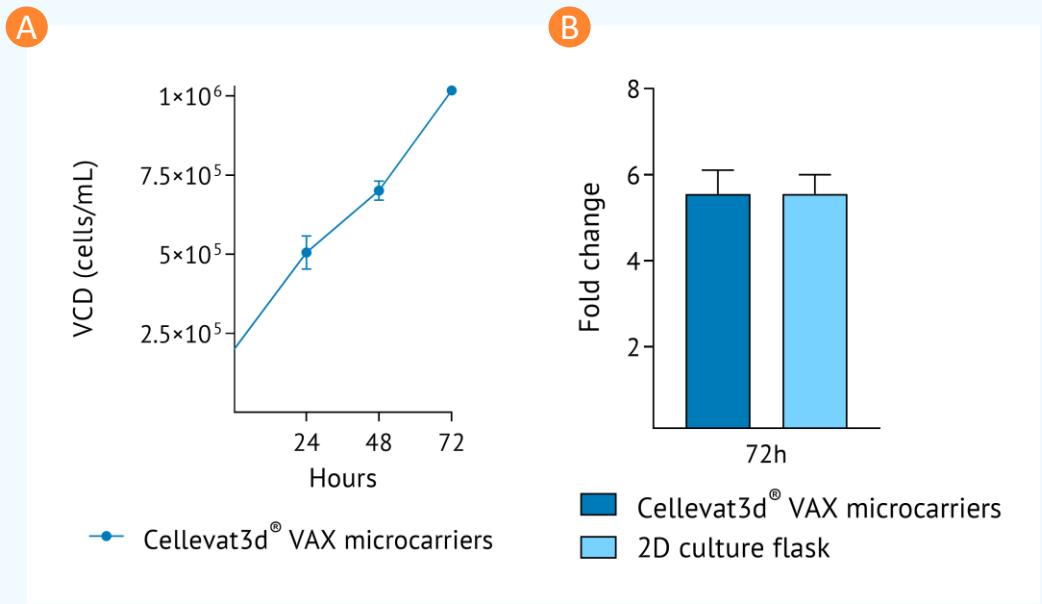
HEK 293T cells were inoculated in spinner flasks at a density of $1\text{x}10^6$ cells/mL of final culture volume with either sterilized Cellevat3d® nanofiber microcarriers (0.075 mg/mL) or sterilized industry standard microcarriers (0.96 mg/mL) in DMEM with 10% FBS, working volume 75mL. The cultures were agitated at 25 rpm for 3h, followed by agitation at 60 rpm on a magnetic stirrer placed inside humidified incubator at 37°C with 5% CO_2.

When the 3D nanofiber and standard microcarrier cell cultures reached cell densities of $0.8\text{ x}10^6$ cells/mL and $1.1\text{-}1.3\text{x}10^6$ cells/mL, respectively, they were infected with enterovirus at a MOI of 0.0001. Samples were taken 3 days post-infection, and infectious virus titers were determined by the $TCID_{50}$ assay (**Ref 3**) to compare virus production between the two approaches.

Results

1. Homogenous high cell density 3D spheroid cultures of Vero cells using Cellevat3d® VAX nanofiber microcarriers

The data showed that Vero cells growing on Cellevat3d® VAX nanofiber microcarriers formed homogeneous 3D spheroid cultures, reaching a viable cell density of $1x10^6$ cells/mL after 72h in culture and similar fold change relative seeding density as parallel 2D cell culture flasks (**Figure 1A-D**).



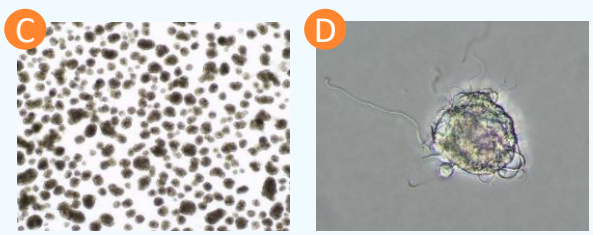


Figure 1. Vero cell growth using Cellevat3d® VAX nanofiber microcarriers **A)** Vero cell growth curve and **B)** fold change relative seeding density for Cellevat3d® VAX and 2D cell cultures after 72h of cell growth. Brightfield microscope images of 3D spheroids at **C)** 4x magnification and **D)** 40x magnification.

2. Successful production of enterovirus A71 using Cellevat3d® VAX nanofiber microcarriers

Vero cells expanded on Cellevat3d® VAX nanofiber microcarriers in 3D spheroid culture were successfully infected with EV71, resulting in the production of functional viruses capable of infecting new cells. There was a positive correlation between infectious virus titers measured on day 8 post-infection and MOI (**Table 2**). Maximum titers of $8.33x10^6$ $TCID_{50}$ per mL were reached at a MOI of 0.01 which compared well to the virus titer of the EV71 seed lot obtained from the supplier (ATCC, $8.9x10^6$ $TCID_{50}$ per mL) demonstrating the functionality of our novel nanofiber-based approach even in a non-optimized cell culture conditions.

Day 8 post-infection	$TCID_{50}$ per mL
MOI^{-2}	$8.33x10^6$
MOI^{-3}	$1.98x10^5$
MOI^{-4}	$1.11x10^5$

Table 2. Infectious virus titers ($TCID_{50}$ per mL) of EV71 produced by Vero cells grown on Cellevat3d® VAX nanofiber microcarriers, infected at different MOIs and measured on day 8 post-infection. Titers were calculated using the Spearman-Kärber method (**Ref 4, 5**).

3. 11.4-fold higher infectious enterovirus (non-disclosed) titers using Cellevat3d® nanofiber microcarriers

Benchmarking data generated by Intravacc (CDMO), demonstrated that HEK 293T spheroid cell cultures grown on nanofiber microcarriers produced 11.4-fold higher infectious titers of enterovirus ($5.01x10^6$ $TCID_{50}$ per mL) compared to industry standard microcarrier cell cultures ($4.41x10^5$ $TCID_{50}$ per mL) (**Figure 2**). Additionally, the nanofiber spheroid cultures achieved a cell-specific virus yield (CSVY) of 6.26 $TCID_{50}$ per cell, representing a 17-fold increase over standard microcarrier cultures, which yielded 0.37 $TCID_{50}$ per cell.

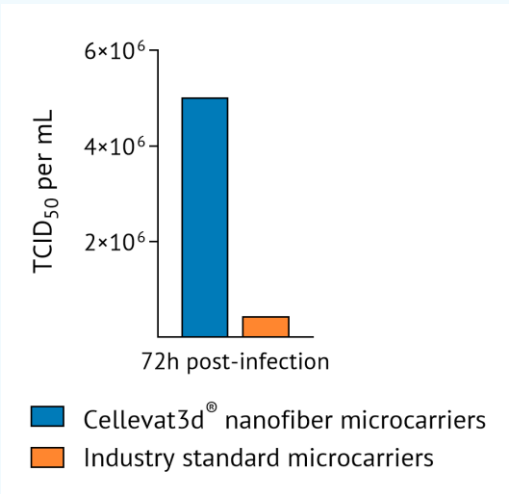


Figure 2. The HEK 293T cells grown on either Cellevat3d® nanofiber microcarriers or industry standard microcarriers in replicate flasks were infected with enterovirus at a MOI of 0.0001, and the viral titers were measured three days post infection.

Conclusions

Cellevat3d® VAX nanofiber microcarriers provide a sustainable and scalable solution that significantly enhances productivity in viral vaccine manufacturing. Our results demonstrate robust Vero cell growth in the format of cell-dense 3D spheroid cultures, which efficiently produced high-quality, infectious EV71 virus particles.

Importantly, benchmarking data from Intravacc (an independent CDMO) showed that HEK 293T cells cultured on Cellevat3d® nanofiber microcarriers and infected with a different enterovirus strain yielded more than a 10-fold increase in virus titer and volumetric productivity compared to conventional bead based microcarriers processes.

Altogether, the Cellevat3d® nanofiber-based products can be rapidly and seamlessly integrated into existing stirred tank bioreactors and process development workflows, offering a novel and high-performance solution for improved vaccine productivity and reduced cost of production.

Acknowledgements

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