scale-X™ bioreactor for viral vector production

Proof of concept for scalable and homogeneous Vero cell growth

Application note

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Abstract

The scale-X™ bioreactor is a novel fixed-bed system that enables cultivation of surface-dependent and suspension cell lines. A large surface area in small-footprint units enable high cell concentrations. This study compared adherent Vero cell culture in scale-X hydro bioreactors with standard microcarrier cultivations. Both systems enabled similar surface-specific cell concentrations. Subsequent unwinding of the spiral-wound fixed-bed revealed a uniform cell distribution in axial and radial directions. This indicates a homogeneous fluid flow and cell attachment inside the bioreactor. The observed homogenous cell growth is an important scaling factor and allows maximum use of available surface area in a reproducible manner.

Introduction to the scale-X bioreactor

Most viral vaccines manufacturing processes are based on surface-dependent cell platforms. Typical cultivation systems such as roller bottles, multi-tray cell culture dishes and microcarrier bioreactors have constraints for large-scale production. To overcome potential scale-up limitations and to ease manufacturing, fixed-bed bioreactors are a valuable alternative. The innovative scale-X bioreactor is a scalable fixed-bed system from research- (2.4 m^2 of growth surface) to pilot- (10 and 30 m^2 of growth surface) and manufacturing-scale (200 and 600 m^2 of growth surface; Fig. 1). In comparison to randomly packed polyethylene terephthalate (PET) fabric strips, the proprietary scale-X technology uses a structured, spiral wound PET fixed-bed to reduce local over compaction and dead zones, while making optimal usage of available surface area with high batch-to-batch reproducibility (Fig. 2).

Materials, Methods & Equipment

Fixed-bed process: scale-X hydro bioreactor

Cell line and process conditions

Cell line	Vero ATCC® CCL-81.5TM
Seeding concentration	1×10^4 cells/cm^2
Reactor volume	700 mL
Fixed-bed volume	170 mL
Fixed-bed surface area	2.4 m^2
Working volume	2700 mL
Stirring speed	740 rpm

Method for evaluation of cell concentration and distribution

- Twelve incorporated sample strips (each 12.15 cm^2) were extracted from the fixed-bed and well mixed for 3 min in 1 mL cell lysis buffer. Released nuclei were stained using crystal violet and manually counted.
- When the targeted cell concentration is reached, the fixed-bed was removed, carefully rolled out and defined areas of 1 cm^2 were cut (Fig. 3). Cells from the strip were lysed with reagent A100 (ChemoMetec) and the nuclei were counted with a hemocytometer.

Figure 1: scale-X bioreactor range from hydro (2.4 m^2 for R&D) to carbo (10 and 30 m^2 for pre-clinical) and nitro (200 and 600 m^2 for commercial).

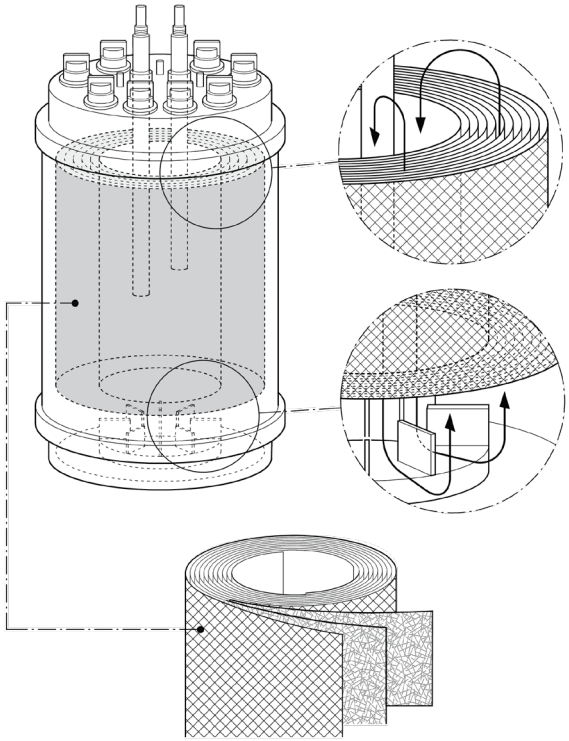

Figure 2: Proprietary fixed-bed structure and media flow path in the scale-X bioreactor series.

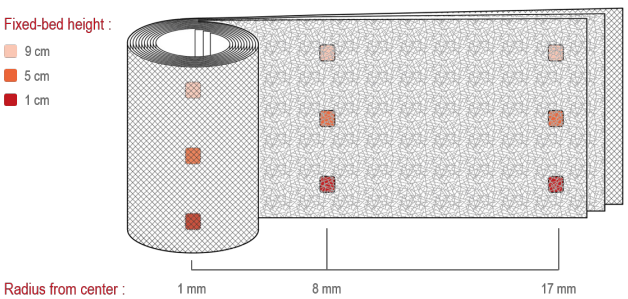

Figure 3: Sampling points for cell concentration measurements in the scale-X fixed-bed.

Reference process: Cytodex® 1 microcarriers in stirred-tank bioreactor (STR)

Cell line and process conditions

Cell line	Vero ATCC® CCL-81.5
Seeding concentration	2×10^4 cells/cm²
Reactor volume	850 mL
Microcarrier concentration	2 g/L Cytodex® 1
Microcarrier surface area	0.75 m²
Working volume	850 mL
Stirring speed	90 rpm

Method for cell concentration measurement

- A sample volume of 5 mL was collected from the bioreactor. After three washing steps, cells were disrupted with a 0.1 M citric acid, 0.1% crystal violet and 0.1% Triton X-100 solution.
- After an incubation time of 1 h at 37°C, stained nuclei were counted with a hemocytometer. The method is described in detail by Trabelsi et al. (Khaled Trabelsi, 2004)

Process parameters (for both cultivations)

- Serum-free medium (VP-SFM, Gibco™) supplemented with 4 mM glutamine
- Feeding of glucose and glutamine stock solutions to prevent substrate limitation below 8 mM and 2 mM, respectively
- pH control at 7.2 with CO^2 and 0.5 $NaHCO^3$ (microcarrier) or 0.5 NaOH (scale-X)
- Temperature set point at 37°C
- Dissolved Oxygen set point at 50% (headspace aeration)

Vero cell growth

Comparison of maximum Vero cell concentrations in different bioreactor systems (Kiesslich, 2020) (Fig. 4):

scale-X hydro bioreactor (2.4 m²): 2.7×10^5 cells/cm²
STR Cytodex® 1 (0.75 m²): 2.4×10^5 cells/cm²

Different cell seeding concentrations resulted in shifted but comparable growth profiles. Surface-specific cell concentrations (Fig. 4) were slightly higher in the scale-X bioreactor (2.7 x 10^5 cells/m²) compared to the microcarrier cultivation (2.4 x 10^5 cells/m²). Both systems allowed a similar total medium usage with $2.1–2.2\times10^9$ cells/L_{medium}. When considering the reactor volume of both systems at equivalent throughput, the fixed-bed bioreactor represents a strongly reduced equipment footprint (5-fold reduction).

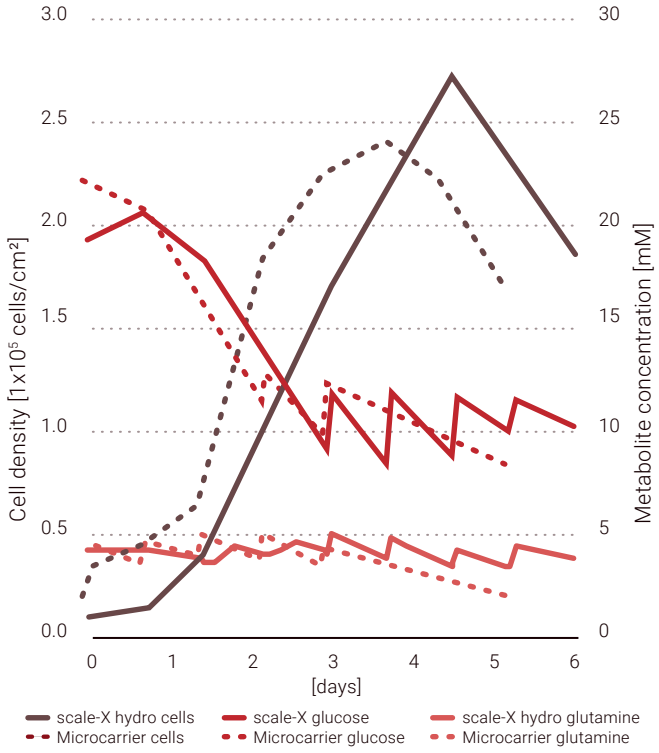

Figure 4: Vero cell growth in a scale-X hydro bioreactor compared to a standard microcarrier cultivation, kindly provided by Kiesslich et al. (2020).

An extended Vero cell growth study was done at Univercells (Fig. 5). Multiple experiments were performed in the scale-X hydro bioreactor (n=26) resulting in reproducible cell concentrations of an average of 2.5×10^5 cells/cm². The scale-up to the larger scale-X carbo bioreactor (10 m² and 30 m²) resulted in similar surface-specific cell concentrations

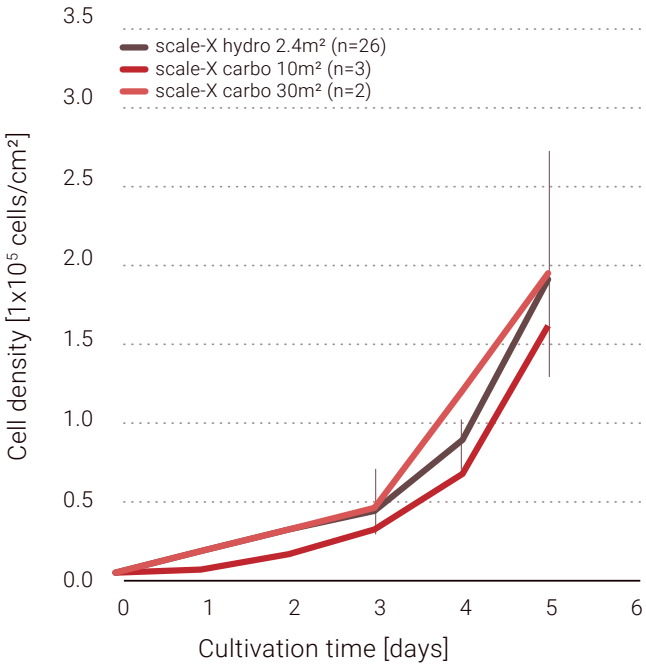

Figure 5: Repeated growth studies with Vero cells in different scale-X bioreactor systems, performed at Univercells.

Vero cell distribution in a structured, spiral wound fixed-bed

Vero cell distribution was investigated at different stages of thecultivation (at 2.7×105 cells/cm2 and 5.3×105 cells/cm2, Fig. 6).Therefore, nine samples were extracted from the unwound fixedbedand cell concentrations were determined.Vero cells in the scale-X hydro bioreactor were homogeneouslydistributed among the fixed-bed, both in axial and radial direction.Additional computational fluid dynamic studies were performedand indicated the absence of dead zones and inhomogeneitiesin the structured fixed-bed (data not shown). Uniform cell growthenabled the full usage of available surface area

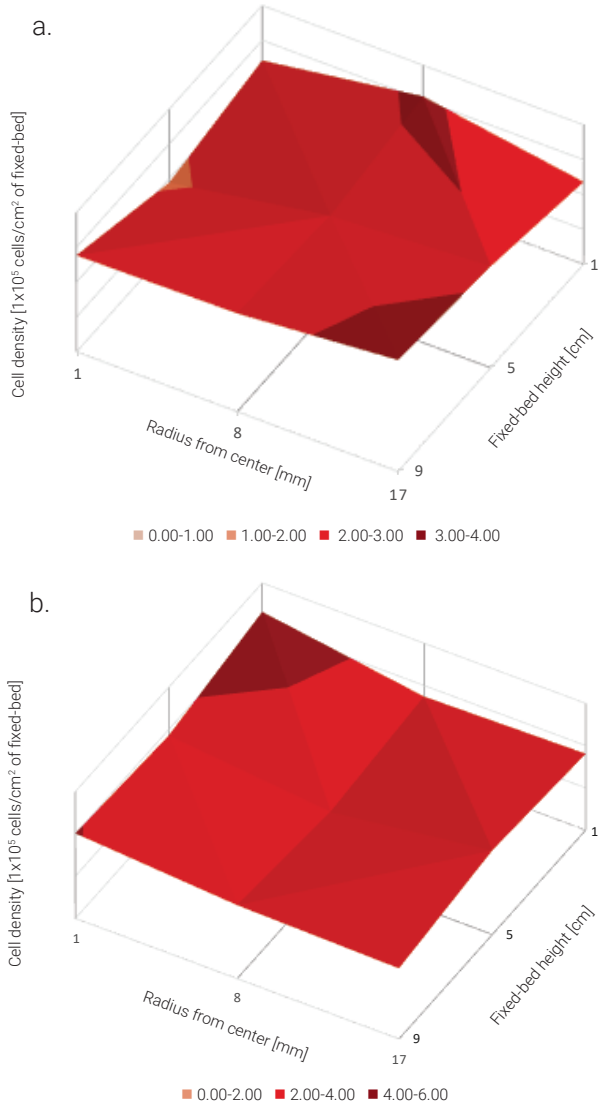

Figure 6: Vero cell growth distribution in the structured fixed-bed of scale-X bioreactors. Cell distribution among the radial (radius from center) and axial direction (fixed-bed height) is homogeneous. (a.) Kindly provided by Kiesslich et al. (2020), (b.) Performed at Univercells.

Conclusion and perspectives

Adherent Vero cells reached equal concentrations in bothprocesses. When transferred to larger fixed-bed scale-Xbioreactors, similar cell growth profiles and maximum surfacespecificcell concentrations were achieved. Finally, studies onthe Vero cell growth distribution revealed a homogenous cellattachment and growth within the fixed-bed. The compactdesign of the fixed-bed allows a reduction in equipmentfootprint by factor 5 compared to the microcarrier process.Overall, results show that the structured scale-X fixed-bedbioreactor system is a suitable alternative to traditional "scaleout"technologies such as multi-tray dishes and roller bottles,but also to technically challenging cultivation systems likemicrocarrier processes.The current scale-X bioreactor can be transferred tomanufacturing scale (600 m2) and integrated into a continuousNevoLine™ manufacturing platform. The NevoLine systemcouples intensified upstream processing to continuousdownstream processing to allow low-cost, large-scalecapacity with reduced capital and operating costs.

Bibliography

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