Scalable, high performance single-use bioreactor technology for viral vectors production

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Abstract

Adherent cells for the production of viral vectors are widely-used in the development and commercialization of gene therapies. Traditional industrial processes use static methods such as multi-tray plasticware, but these suffer from a number of limitations: they lack precise environmental control (pH, DO, media composition), are heavily dependent on manual operations and can only be scaled-out as opposed to scaled-up. The need for high-performance, automated and scalable production tools is two-fold: gene therapies typically require large doses of active viral agents per patient, and the global demand for the latter is expected to rise significantly in the near future. Univercells has developed a scalable fixed-bed bioreactor technology which offers to meet high capacity demands in an affordable manner.

1. scale-X™ single-use fixed bed bioreactor system

The scale-X system is a high-performance fixed-bed bioreactor designed for the cost-effective and scalable culture of adherent cells and viral production.

Featuring advanced technology that allows high cell density per unit volume as well as consistent homogeneity, scale-X delivers high titers and high quality viral production, while reducing deviation risks through a robust process control system.

The modularity and linear scalability of the scale-X product range, ensures a smooth transition from R&D to clinical stages and full scale industrial production.

The scale-X system presents several features of interest suited for the high-performance production of gene therapies:

Figure 1: scale-X™ bioreactor system

Key features

- Low-footprint, high surface area
- Linear scalability
- Optional automated in-line concentration of product
- Cell sampling
- Single-use (autoclavable or gamma-irradiated)
- Pre-assembled tubing manifolds
- R&D to clinical batches in one system
- Ultra-low shear stress

Figure 2: construction, flow and cell attachment principle

2. Process setup

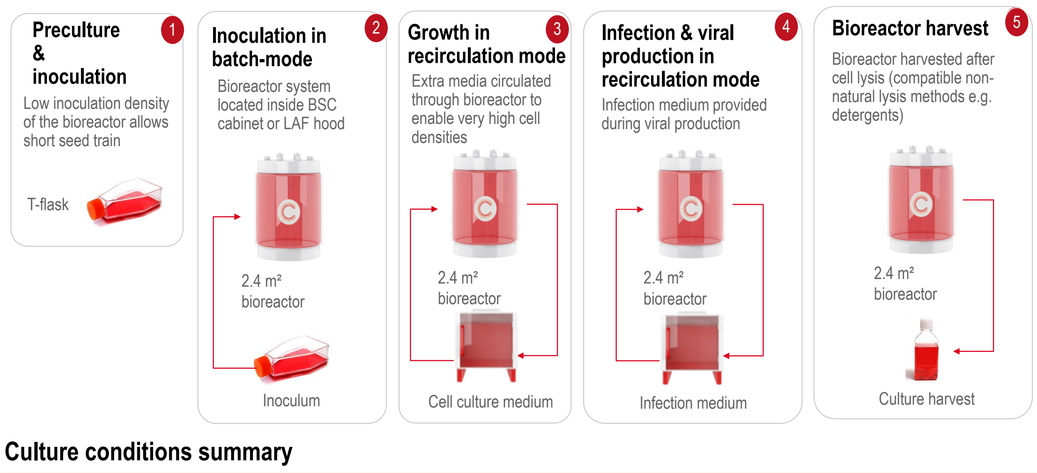
> Bioreactor preparation: the bioreactor fixed bed was hydrated with culture medium under circulating conditions (1 cm s^{-1} linear velocity, 1 hr total time). Medium equilibration was achieved at the experimental set points (37°C, pH 7.2). DO calibration was achieved under non-regulated aerated conditions (100% set point) before starting regulation (>50%).

> Inoculation: the bioreactor was inoculated at a seeding density of 3.0 x 10^4 cells cm^{-2} under agitated conditions. Cell attachment was verified by measuring the turbidity of the supernatant 2 hours post-inoculation.

> Cell expansion: in all experiments shown here an external media source (4.2 L) was connected shortly after inoculation.

> Product harvest: depending on the cell lysis method, .

Figure 3: cell culture & infection process steps



Culture conditions summary

> Bioreactor vessel and effective working volume:	scale-X hydro, 750 mL
> Cell type	Vero, HEK293
> Agitation speed:	740 rpm (1 cm s^{-1} vertical velocity)
> Innoculation density:	30,000 cells cm^2
> pH:	7.2
> Dissolved oxygen:	50%
> Culture time:	3 – 6 days
> Cell density estimation	Nuclei count on carriers by crystal violet staining

3. Transfer of cell culture from multi-tray system to bioreactor

Cultivation of Vero cells in medium with serum was carried out in 2.4m² compact fixed-bed bioreactors achieving high cell density. Cell dispersion results show homogeneity in the fixed bed, ensuring even and predictable growth throughout the structure (figure4). Thanks to its unique compact structure, scale-X™ bioreactors seeded with cell densities a quarter of that needed for traditional systems grow nearly 50-fold to reach a final concentration of 200,000+cells/cm²(figure3).

Figure 4: Fixed-bed vertical dispersion in the scale-X™ hydro bioreactor system

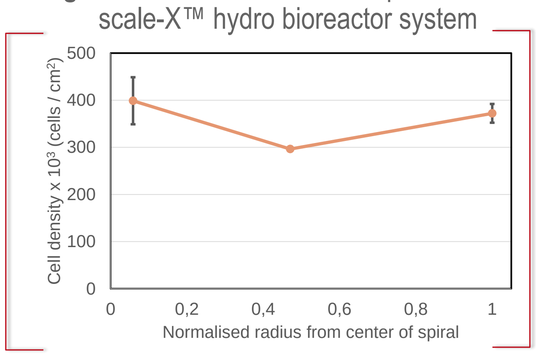
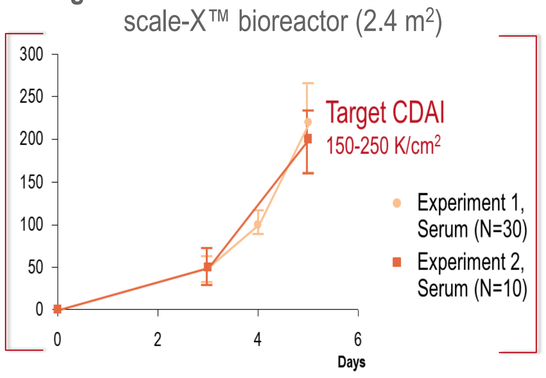


Figure 5: Growth of Vero cells cultivated in scale-X™ bioreactor (2.4 m^2)



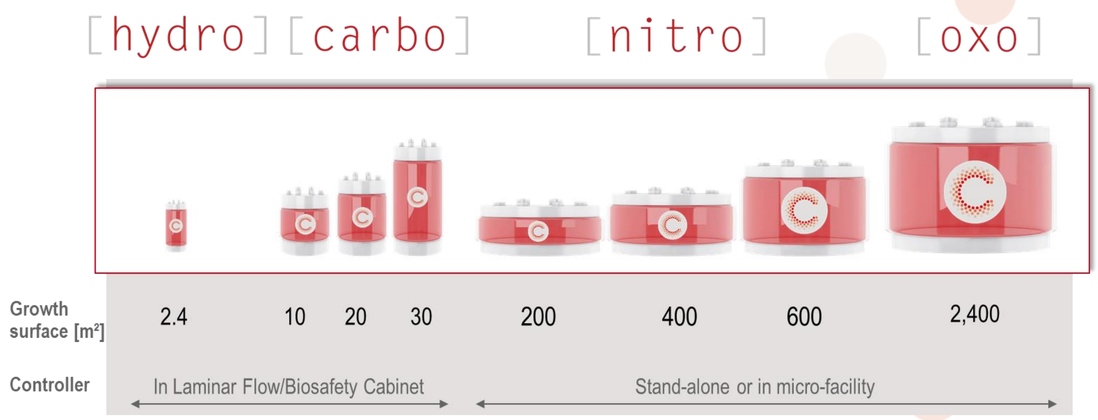
4. Viral productivity

The processes developed thus far for HEK293 and Vero cells producing viruses and viral vectors achieve promising productivity and yields. The overall processes achieve high productivity, delivering an equivalent of 50 doses for one therapy per scale-X hydro bioreactor (2.4 m^2). The following data shows an extrapolation of the yields in larger scale systems and their equivalence in multi-tray plastic ware:

Table 1: extrapolated doses per bioreactor and multi-tray plastic ware for comparison

	# doses	Multi-tray plasticware (40 stacks) per 1 bioreactor
1 x scale-X™ hydro	50	1
1 x scale-X™ carbo	640	12
1 x scale-X™ nitro	12,700	240
1 x scale-X™ oxo	50,900	950

Figure 6: scale-X™ bioreactor range at R&D, pilot and industrial scales



5. Conclusion

> Successful transfer of cell culture from static plasticware to fixed bed bioreactor

> Very high cell densities observed in bioreactor due to better environmental control

> Promising viral yields and scope for determination and optimization of Critical Culture Parameters

> Due to its intrinsic scalability, the scale-X bioreactor offers encouraging prospects in achieving high-capacity production of viral vectors at larger scale, for a smooth scale-up of gene therapies in an affordable manner

6. Perspectives

> Based on the successful results at 2.4 m^2 scale, the next step will focus on scale-up work to pilot and production scales (scale-x carbo and nitro), and will also integrate the following:
  - Application to a wide range of viral vectors and oncolytic viruses
  - Application in transfection processes

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