Cultivation of Vero Cells to High Cell Densities for Human Influenza Vaccine Production

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Introduction

Annual vaccination is an effective method to prevent influenza infection. However, catering to the need of influenza vaccines in developing countries faces many challenges. Timely production and deployment of influenza vaccines is required in non-vaccine producing countries. We focus on the rapid development of influenza vaccine with the goal of generating a perfect bioprocessing solution as an alternative to chicken eggs that can aid to speed up the manufacturing process.

Esco Aster's vision

Esco Aster focuses on high-quality, biomanufacturing of vaccines, biologics, and cell-therapy products. To enable our primary vision to help non-vaccine producing countries to be self-sufficient in manufacturing, storing, and distribution of vaccines, we recently partnered with biotech company Nuvonis (Austria) to establish efficient bioprocessing workflows. It will enable the generation of influenza virus using Nuvonis serum-free Vero cell banks. We demonstrate that the Tide Motion manufacturing platform, modularly integrated with Esco Cell Processing Isolator, helps to localize vaccine production, making it more affordable - in terms of CAPEX & OPEX - for developing countries

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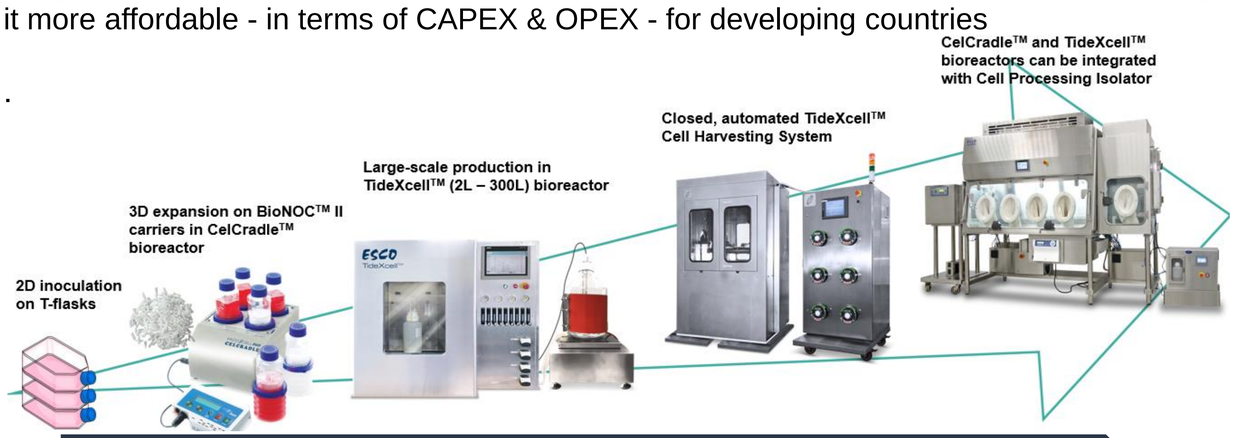


Tide Motion Platform + Cell Processing Isolator

Vero Cells

Vero cells are anchorage-dependent cells that are widely used for vaccine production. They are derived from the epithelial kidney cells of African Green monkeys. They have many advantages in terms of high viral infectivity and thus are very effective for primary virus isolation. The array of viruses that Vero cells are susceptible to and the various Viral Vaccines that can be produced in these cells are Influenza Rabies, Reovirus and Japanese Encephalitis virus to name a few.

Considering the multitude of advantages of Vero cells, it is desirable to cultivate Vero cells to a high density to optimise virus production. The cells are conventionally grown in T-flasks and Roller bottles in 2D culture. We culture Vero cells to an optimal density using macrocarriers – BioNOC™ II – which form the heart of the Bioreactor technology.

Vero Cell Cultivation in Tide Motion Bioreactors

A serum-free Vero cell research cell bank (passage 144-160) is used for all applications. The cell banks – both MCB and WCB – have been fully characterized including tumorigenicity testing at the end of production level (EOP). Serum-free media was supplemented with L-glutamine before use. A serum-supplemented media was also used in cell growth assays intended to optimize cell densities. Glucose in the media was measured using a GlucCell™ device and Esco VacciXcell GlucCell™ Glucose Test Strips. The growth kinetics are displayed in Figure 1 (a) and (b) as below. The Glucose consumption and Glucose utilization rate are shown in (c) and (d)

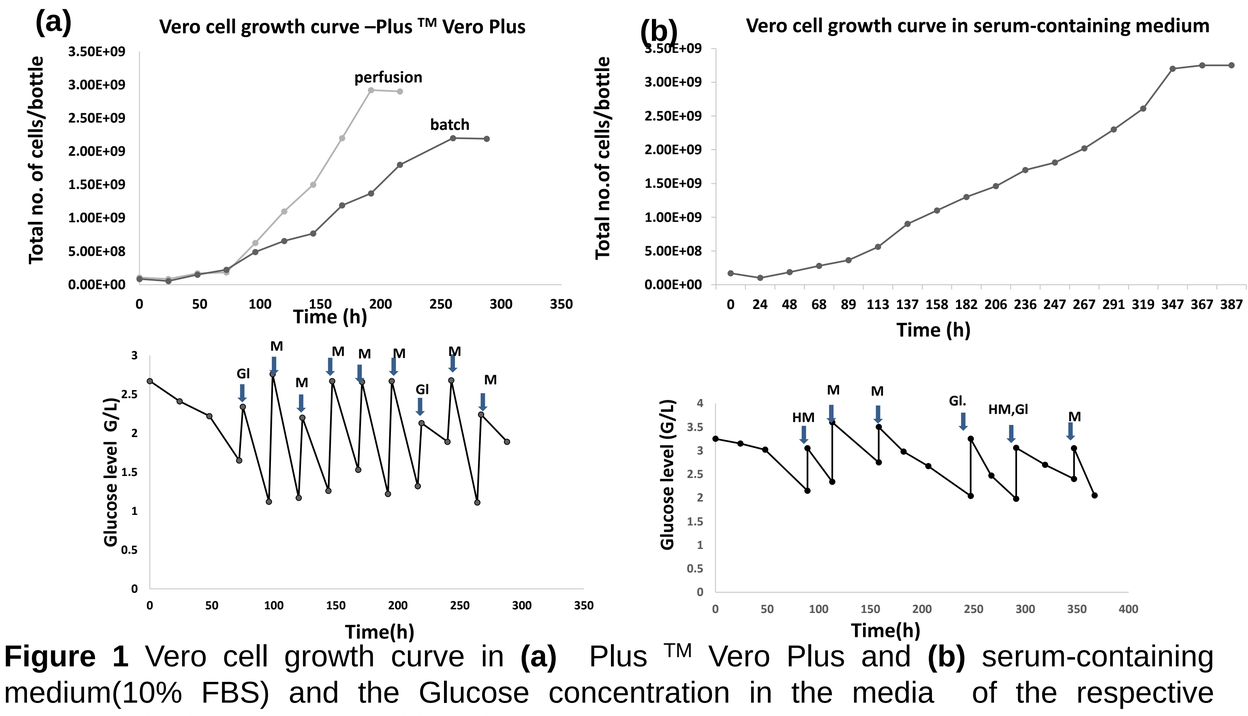


Figure 1 Vero cell growth curve in **(a)** Plus ™ Vero Plus and **(b)** serum-containing medium(10% FBS) and the Glucose concentration in the media of the respective cultures. M=Media change; HM=half media change; Gl=Glucose

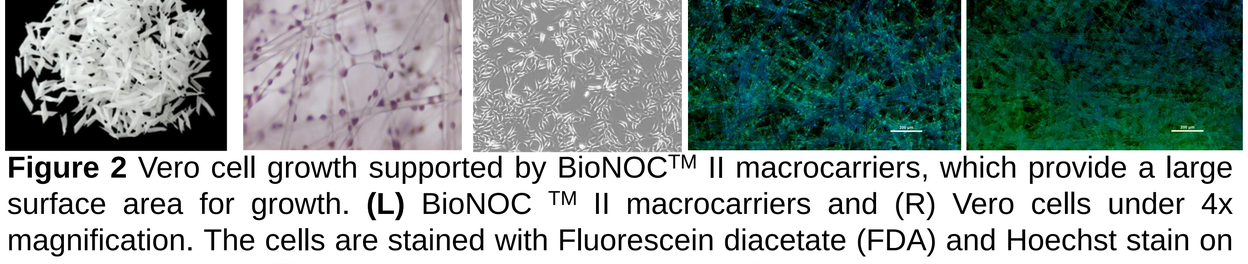
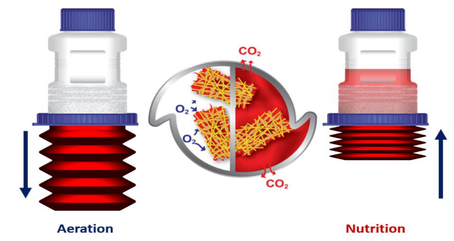


Figure 2 Vero cell growth supported by BioNOC™ II macrocarriers, which provide a large surface area for growth. **(L)** BioNOC ™ II macrocarriers and (R) Vero cells under 4x magnification. The cells are stained with Fluorescein diacetate (FDA) and Hoechst stain on the extreme right. The left panel is representative of early culture and the right panel represents late culture.

Cultivation of Vero Cells for Influenza Vaccine Production

Cells from five confluent T-175 flasks were harvested by trypsinisation, centrifuged for 5 min at 275 x g, and resuspended in cell growth medium. Cells were mixed with cell growth medium in a total volume of 500 ml and transferred to a CelCradle-500AP™ (perfusion) using the following Tide Motion parameters. After 1 hour and 45 minutes, media was sampled, and cells were counted using the trypan blue dye exclusion test for cell viability. 85% of the cells attached stably to the carriers. Tide parameters for cell growth are as follows:

Uprate	Uphold	Downrate	Downhold
2.0 mm/s	20 sec	2.0 mm/s	0 sec



Virus Infection Method

A total cell number of 1.7e9 cells was achieved at 167 hours (7 days) after seeding of the Vero cells in serum-free condition. The doubling time of Vero cells was 36 hours – similar to cells grown in T-flasks.

Vero cells were infected with an influenza A strain at a desired multiplicity of infection (MOI) of 0.01. Following viral infection, the medium was replaced seven times. Infectious virus titre in each medium fraction was determined by fluorescent focus assay (FFA). In total 10.45e10 FFUs of infectious virus was recovered. The schematic is depicted in Figure 3 and the results are as in Figure 4(a-e)

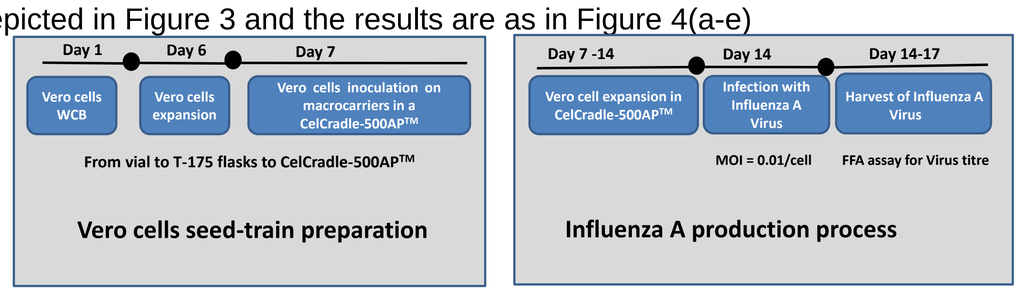
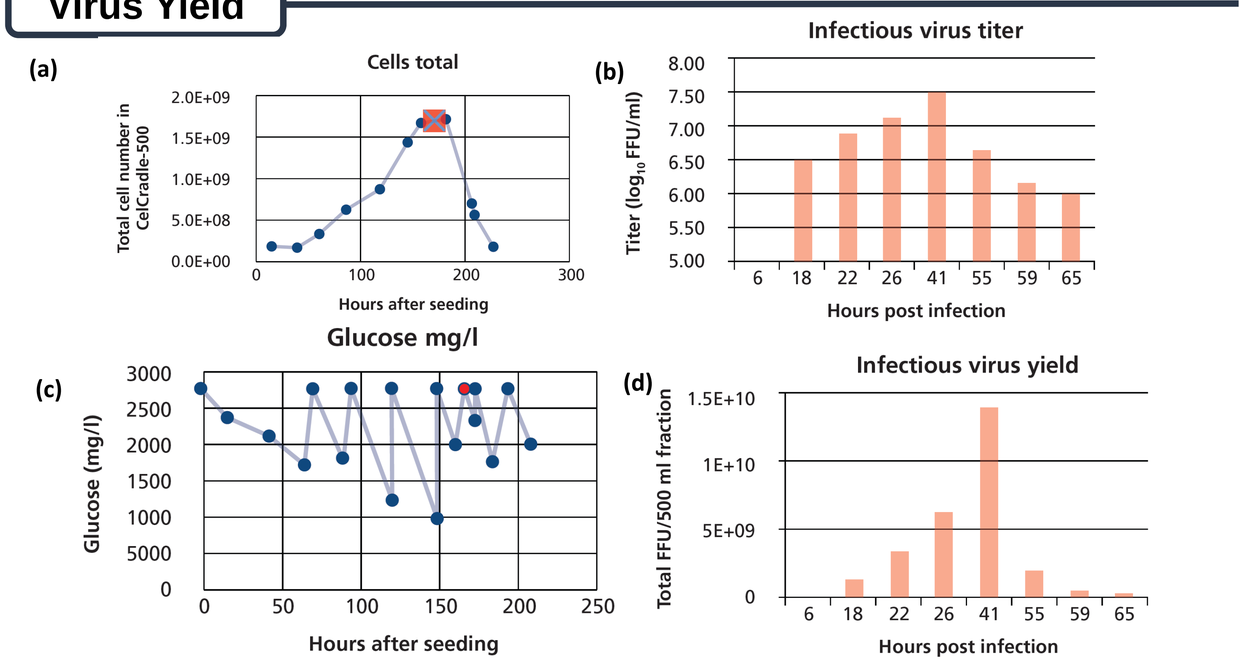


Figure 3 Vero cell seed train preparation and Influenza A production process in the CelCradle-500AP™; WCB - Working Cell Bank; MOI - Multiplicity of Infection ;FFA - Fluorescent Focus Assay.

Virus Yield



(e)

	2D Culture Cell Factories CF10	3D BioNOC™ II carriers
Cell morphology	Mono/bilayer	Densely populated carriers
Cell density	0.7 Million per ml	3.2 Million per ml
Working volume	1.5L	0.5L
Surface area	6.320 cm²	15.000 cm²
To obtain 1.6E9 cells	1.6 x CF10	1 X 500 ml CelCradle

Figure 4 (a) CelCradle-500AP™: Vero cell growth in serum-free medium (SFM). The red square indicates the time of infection at 161 hours after seeding **(b)** Glucose concentration in the media **(c)** Infectious virus titre in log FFU/ml **(d)** Infectious virus yield in FFU per 500 ml fraction **(e)** High cell density was achieved in 3D culturing compared to 2D culturing

Conclusion

In this proof-of concept study, it has been demonstrated that Vero cells can be grown to extremely high densities of 2.9e9 (5e6 cells/ml) to 3.25e9 (6e6cells/ml) per 500ml CelCradle™ using SFM and serum-containing media respectively. This represents an efficient bioprocess workflow of a single-use strategy that can be scaled up for biomanufacturing of affordable Vaccines using the Tide Motion technology. These studies have been conducted by our partner at the Rand D facility located in Austria.

Future Studies

The results are indeed promising. Considering that Vero cells are the "workhorse of the Vaccine industry", other strains of Influenza Viruses/other Virus strains can be efficiently cultured to a high titre using the Tide Motion technology, with potential for a cGMP facility for bulk production of Vaccines for stockpiling or in the event of a disease outbreak.

Although these results are promising, our results can be further improved by an appropriate more elaborate optimization of influenza virus growth in the CelCradle-500AP™ system, potentially in a cGMP facility to scale up for the bulk production of the vaccine.

Esco Aster's Vero pipeline

- Vaccine development
- PD, purification, consultancy for Vaccines
- Transfer of processes,technologies worldwide

Acknowledgements

Our acknowledgements and special thanks are due to to Nuvonis Technologies GmbH, a biotechnology company based in Vienna Austria, for its fully characterized GMP Vero cell line and for production of the Influenza Virus .

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