WHITE PAPER
UPSTREAM BIOPROCESSING

Cultivation of serum-free Vero cells to high cell densities for influenza vaccine production

Background and introduction

Annual vaccination is an effective method to prevent influenza infection. However, expanding the use of influenza vaccines in the Asia-pacific region faces many challenges. Timely production and deployment of influenza vaccines against seasonal and potential pandemic influenza viruses is required in non-vaccine producing countries (many of these still exist in Asia-pacific). In this whitepaper, we focus on the rapid development of influenza vaccine with the goal of generating a perfect bioprocessing solution that can speed up manufacturing process as an alternative to chicken eggs production.

Esco Aster, an independent contract development and manufacturing organisation (CDMO) headquartered in Singapore focus on high-quality biomanufacturing of vaccines, biologics, and cell-therapy products, has a full-range of services and we partner global clients from early-stage discovery to market approvals of 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 that would enable the generation of influenza virus using Nuvonis serum-free Vero cell banks. The Nuvonis Vero cells originated from WHO cells and were adapted to serum-free/ animal component free growth. A Nuvonis working cell bank was used to demonstrate that Esco Vaccixcell CelCradle-500AP can be used to produce high cell densities under serum free condition, without the need of lengthy optimisation.

Summary

Nuvonis Vero cells were cultivated under serum-free conditions in a EscoVaccixcell CelCradle-500 single use bioreactor filled with BioNOC™ II carriers.

A total cell number of 1.7 X 10E9 cells was achieved at 167 hours (estimatedas 7 days) after seeding of the Vero cells in serum-free condition. The doublingtime of Vero cells was 36 hours – this was similar for cells grown inT-flasks.

Vero cells were infected with an influenza A strain at a desired multiplicity ofinfection (MOI) of 0.01. Following viral infection, the medium was replacedseven times. Infectious virus titre in each medium fraction was determinedby fluorescent focus assay (FFA). In total 10.45 log10 FFU/ml infectious virus was recovered.

Media and cells

A serum-free Vero cell research cell bank (passage 144-160) was used. Serum Free Media was supplemented with L-glutamine before use. Glucose in the media was measured using a GlucCell™ device and Esco Vaccixcell GlucCell™ Glucose Test Strips.

A recombinant influenza A model virus was used for this proof-of-concept study. The infectious titre was determined by a Fluorescent Focus Assay.

Seeding and cultivation of serum-free Vero cells in a CelCradle-500

Cells from five confluent T175 flasks were harvested by trypsinisation, centrifuged for 5 min at 275 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.

To support immobilization, the following CelCradle parameters were used:

Rising Rate	Top Holding Time	Down Rate	Bottom Holding Line
2.0 mm/s	20 sec.	2.0 mm/s	0 sec.

After 1 hour 45 min media was sampled, and cells were counted using the trypan blue dye exclusion test of cell viability. As 85% of the cells already had attached stably to the BioNOC™ II carriers, CelCradle parameters were set as follows:

Rising Rate	Top Holding Time	Down Rate	Bottom Holding Line
1.0 mm/s	10 sec.	1.0 mm/s	10 sec.

Media was subsequently replaced according to the timelines table below.

Rising Rate	Top Holding
0	Seeding
71.5	Media exchange
95.25	Media exchange
121.25	Media exchange
148.75	Media exchange
167	Media exchange, sampling and infection
173.5	Media exchange, sampling for infectious titer
189.5	Media exchange, sampling for infectious titer
193.5	Media exchange, sampling for infectious titer
208.75	Media exchange, sampling for infectious titer
213	Media exchange, sampling for infectious titer
217	Media exchange, sampling for infectious titer
233	Media exchange, sampling for infectious titer

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Cell attachment

A total of 235 X 10E6 Vero cells was seeded in a volume of 500 ml cell growth medium.

Cell growth

After a lag phase of approximately 48 hours, in average 0.66 populations doublings per day were observed, corresponding to a population doubling time of 36 hours.

A total cell count of 1.6 X10E9 cells was measured 161 hours after seeding. Then, cells were infected with influenza A model virus.

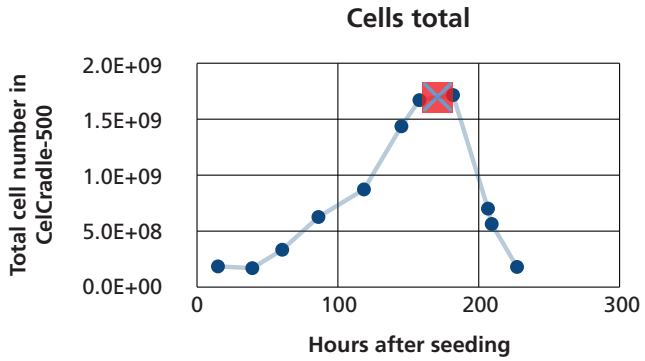

Figure 1:
CelCradle-500AP: Vero cell growth in serum-free OptiPro SFM medium. The red square indicates the time of infection at 161 hours after seeding.

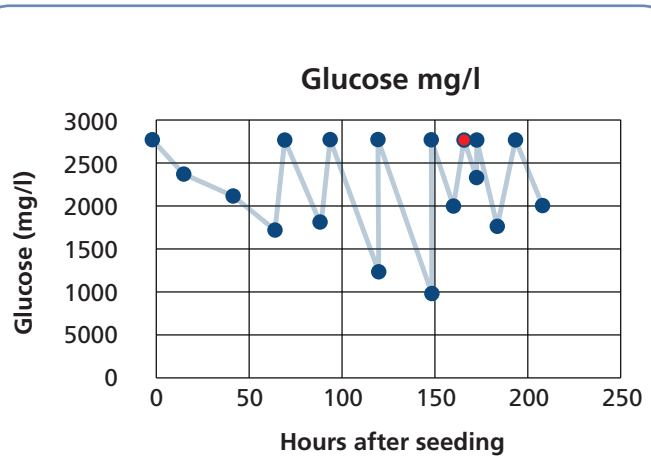

Figure 2:
CelCradle-500AP: Glucose concentration in the media. The red dot indicates the time of infection at 161 hours after seeding.

Virus Yield

Cells were infected with influenza A model virus at an MOI of 0.01. The infection medium was replaced several times. Infectious titre of each medium fraction taken was analysed by FFA assay.

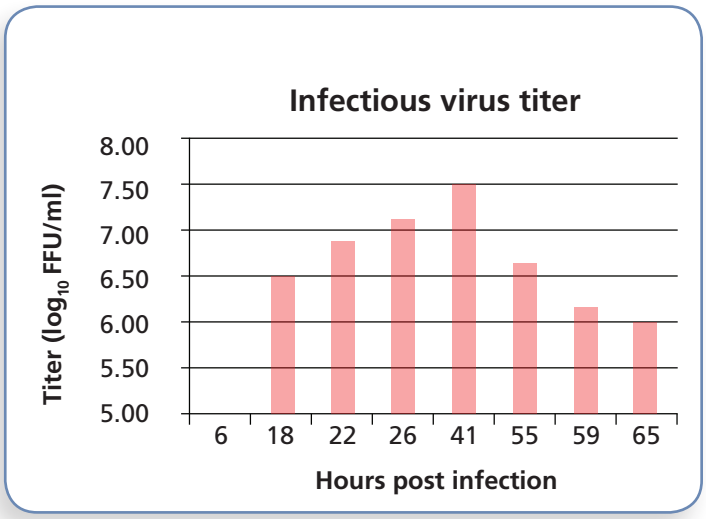

Figure 3:
CelCradle-500AP: Infectious virus titre in log FFU/ml.

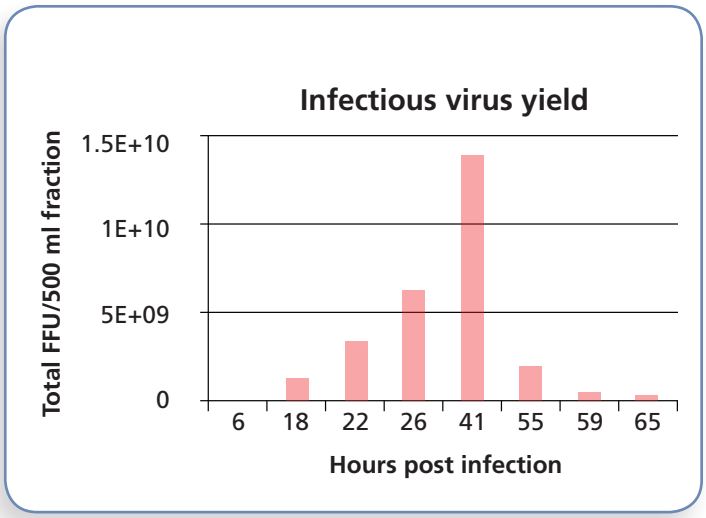

Figure 4:
CelCradle-500AP: Infectious virus yield in FFU per 500 ml fraction.

Virus titre peaked at 7.45 log10 FFU/ml upon 41 hours post-infection. In total 10.45 log10 FFU infectious virus was recovered.

Comparison 2D versus 3D culture

Herein, we provide a comparison of traditional culture methods in 2D and 3D. Our results show a clear difference in achieving high cell density per ml in a densely populated BioNOC™ II carriers.

	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 5:
CelCradle-500AP: high cell density was achieved in 3D culturing compared to 2D culturing

Future studies

In this unoptimized study, we have demonstrated that serum-free Vero cells can be efficiently grown to high cell density in CelCradle-500 in the establishment of bioprocessing workflows of a single-use strategy to enable biomanufacturing of affordable vaccine productions. We have further shown that our partner can replicate the data generated for proof-of-concept study using serum-free Vero cells at their R&D laboratory facility and setup located in Austria.

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

Find out more at
http://www.vaccixcell.com/products-and-brands/celcradle/
http://www.escoaster.com
http://www.nuvonis.com

For enquiries, you may contact
Esco VacciXcell (Bioprocessing Tools): mail@vaccixcell.com
Esco Aster (cGMP CDMO): mail@escoaster.com
Nuvonis (Vero Cell Line Processing): office@nuvonis.com