WHITE PAPER
UPSTREAM BIOPROCESSING

Propagation of Japanese Encephalitis Virus (JEV) in VERO for Vaccine Production Using TideMotion® Bioreactor

Background and Introduction

Japanese encephalitis virus (JEV) was first isolated from the brain of a fatal human case in Japan, 1934. Although symptomatic Japanese encephalitis (JE) is rare, the case-fatality rate can reach as high as 30%. Of those who survive, 30-50% suffer permanent intellect, behavioural or neurological problems. With more than 3 billion people in the South-East Asia and Western Pacific regions potentially at risk of being infected by JEV, vaccination is the best prevention.

Development of vaccines for JE began in the 1940s with formalin-inactivated mouse brain-derived vaccines. Although effective in inducing a protective immune response, the last lot of mouse-derived vaccines was used up in May 2011 because international standards for development of JE vaccines today do not allow for this method of production anymore.

In this whitepaper, we describe the development of a JE vaccine process using a packed-bed cell culture system. We demonstrate a cost-effective bioprocessing solution and a rapid manufacturing process suitable for replacing the mouse brain-derived vaccine.

Overview

Vero cells were cultivated using standard MEM/FBS media in a VacciXcell CelCradle-500AP single use bioreactor filled with BioNOC™ II carriers. A total cell number of $1.2x10^9$ cells was achieved at 96-hour post-seeding. Regarding cell doubling time, cells grown in CelCradle (CC) required ~32 hours while those grown in T-flasks required ~40 hours. This observation clearly demonstrates that TideMotion® technology provides better growing condition for cells, which likely arises from the efficient exchange of nutrients/oxygen between cells and media.

Vero cells were infected with JE virus strain at a desired multiplicity-of-infection (MOI) of 0.01. Following viral infection, a portion of the virus-containing media was harvested daily and replaced with fresh media over seven days of production. Infectious viral titre per harvest was determined using the "gold standard", plaque assay. In total, $1.1x10^{11}$ plaque-forming unit (pfu) infectious virus was generated from *one* CelCradle bioreactor.

Materials

Device	Cell Line/Product	Complete Media	Seed
CelCradle-500AP	Vero Cells/JEV	MEM/10%FBS/ 2mM L-Glutamine	$1.4x10^8$

Table 1: Materials required for performing Phase I – Vero cell expansion.

Phase I: Seeding and Expansion of Vero Cells

Cells from four confluent T175 flasks were harvested by trypsinization and centrifuged for 3 min at 400 x g. $1.4x10^8$ cells were inoculated into 120 ml complete media and transferred to a single CelCradle-500AP bottle with a white cap. Cell seeding was performed by inverting the white-capped CelCradle bottle, which submerges the carriers in the cell slurry. This "inverted cell seeding" was performed in a 37°C CO_2 incubator with gentle swirling every 15-30 min for 3 hours. A high seeding efficiency of 99.51% was achieved after 1 hour. To allow expansion of attached cells, the bottle was topped up with 500 ml of complete media, attached to a 2.2 L perfusion bottle and placed onto the platform with parameters set as below:

CelCradle Volume	TideMotion Parameters	Growth Period
500 ml	Uprate: 1 mm/sec Uphold: 10 sec Downrate: 1 mm/sec Downhold: 10 sec	96 hours (4 days)
Perfusion Bottle Volume	**Perfusion Parameters**	
2200 ml	Pump 1 Volume: 1999 ml Cycles/Day: 24 Schedule: 1111111	

Table 2: Overview of CelCradle stage growth and perfusion parameters for Phase I: Vero cell expansion.

Carriers were sampled every day to perform: (1) CVD count and; (2) visual observation of cell growth and distribution across the matrix using methanol/trypan-blue staining.

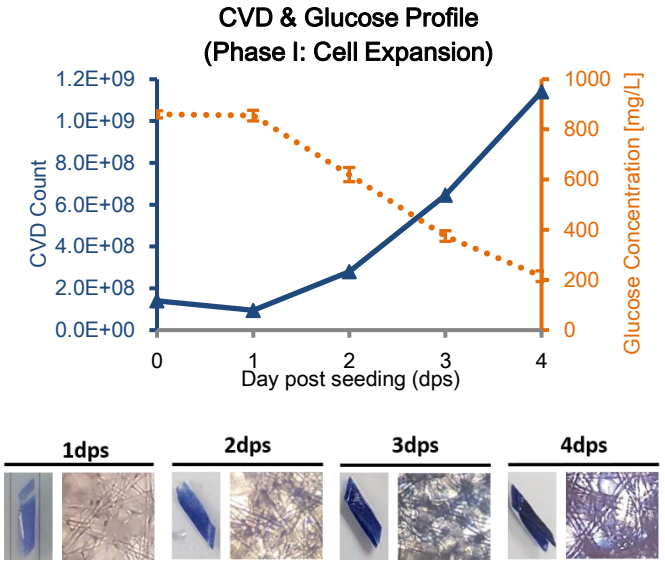


Figure 1: (Top): Cell count were performed daily using CVD with at least 10 randomly picked carriers from CelCradle-500AP bottle. (Bottom): 3 carriers were also methanol fixed, stained with 1x trypan-blue stained for 5 min at RTP then PBS rinsed twice before observing under microscope.

Phase II: Viral Inoculation and Propagation

Cells were infected with JEV at a MOI of 0.01 and a total volume of 500 ml. Virus inoculation and propagation across seven days were performed using parameters shown below:

Time Length	TideMotion Parameters
Phase IIA: Virus Inoculation	
Day 0 (1.5 h)	Uprate: 1 mm/sec Uphold: 15 min Downrate: 1 mm/sec Downhold: 10 sec
Phase IIB: Virus Propagation	
Day 0, 1, 2, 3	Uprate: 1 mm/sec Uphold: 10 sec Downrate: 1 mm/sec Downhold: 10 sec
Day 4	Uprate: 1 mm/sec Uphold: 10 sec Downrate: 1 mm/sec Downhold: 1 min
Day 5, 6, 7	Uprate: 1 mm/sec Uphold: 0 sec Downrate: 1 mm/sec Downhold: 2 min

Table 3: Overview of CelCradle stage parameters for performing Phase II.

Media containing virus product was harvested and replaced with fresh media at different time points across the seven days:

Time Length	Action
Day 0 (1.5 h)	Virus inoculation
Day 0	Virus propagation
Day 1	No harvest; Sample supernatant for viral titre; Suppl. with extra glucose
Day 2	Harvest 470 ml; Replace with fresh 470 ml complete media; Suppl. with extra glucose/glutamine
Day 3	Harvest 470 ml; Replace with fresh 470 ml complete media; Suppl. with extra glucose/glutamine Adjust CO_2 incubator 5 % to 0 %
Day 4	Harvest 470 ml; Replace with fresh 470 ml complete media; Suppl. with extra glucose/glutamine; Suppl. with sodium bicarbonate for pH
Day 5	Harvest 470 ml; Replace with fresh 470 ml complete media; Suppl. with extra glucose/glutamine; Suppl. with sodium bicarbonate for pH
Day 6	Harvest 470 ml; Replace with fresh 470 ml complete media; Suppl. with extra glucose/glutamine; Suppl. with sodium bicarbonate for pH
Day 7	Harvest 500 ml; END

Table 4: Overview schedule of harvest and fresh media replenishment timepoints during the seven days of Phase II.

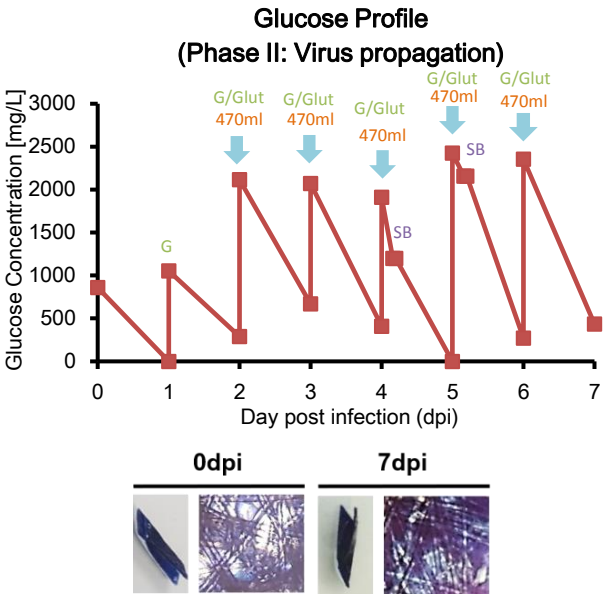


Figure 2: (Top): Glucose concentrations were measured daily using GlucCell meter. Blue arrow represents a supernatant harvest (470 ml) with similar amount of fresh media replaced into bottle. Replacement media were supplemented with glucose [G; green font] and/or glutamine [Glut; green font] and/or sodium bicarbonate [SB; purple font]. (Bottom): 3 carriers randomly picked from CelCradle bottle on 0dpi and 7dpi. Carriers were methanol fixed, stained with 1x trypan-blue stained for 5 min at RTP then PBS rinsed twice before observing under microscope.

Phase III: JEV Quantification Using Plaque Assay

Harvests of virus-containing supernatant were collected daily from Day 2 to 7 (post infection). Supernatant harvests were frozen at -80°C until all harvests were completed. Virus titre was analysed using the "gold standard" plaque assay and using published protocols.

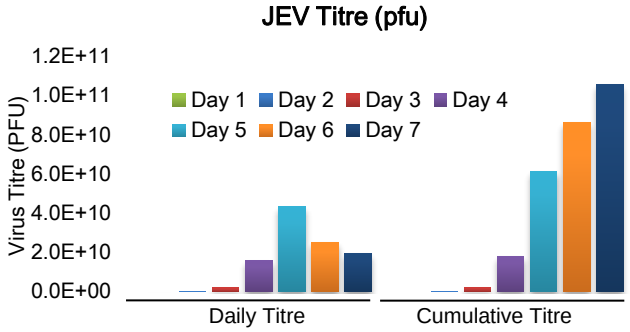


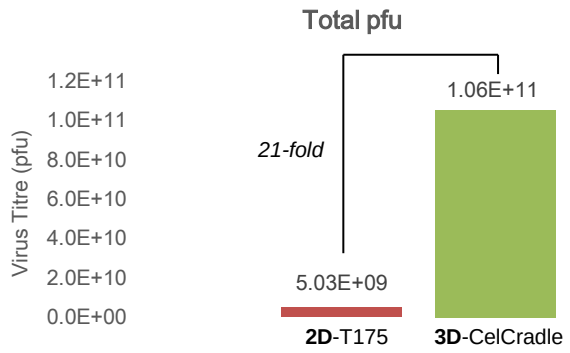
Figure 3: CelCradle-500AP. Titre (infectious JEV) in plaque forming units (pfu).

Harvest Time	Harvest Volume	Harvest Titre	Cumulative Titre	Overall Titre
Day 1	0 ml	-	-	
Day 2	470 ml	$1.63x10^{8}$	$1.63x10^{8}$	
Day 3	470 ml	$1.84x10^{9}$	$2.01x10^{9}$	
Day 4	470 ml	$1.57x10^{10}$	$1.78x10^{10}$	**$1.06x10^{11}$**
Day 5	470 ml	$4.35x10^{10}$	$6.12x10^{10}$	
Day 6	470 ml	$2.51x10^{10}$	$8.63x10^{10}$	
Day 7	500 ml	$1.93x10^{10}$	$1.06x10^{11}$	

Table 5: CelCradle-500AP. Titre (infectious JEV) in plaque forming units (pfu).

Comparison of 2D versus 3D Virus Propagation

Virus propagation using **2D** T-175 flasks and a **3D** CelCradle was compared. JEV production from *one* CelCradle bottle is equivalent to that produced in *twenty-one* flasks of T175.



	CelCradle	T175
Surface Area (cm^2)	approx. 5000	175
Total Virus	$1.06x10^{11}$	$5.03x10^{9}$
Virus per cm^2	$2.1x10^{7}$	$3.0\ x10^{7}$
pfu equiv. to T175	21	1

Discussion

Applying TideMotion technology in cell culture bioreactor ensures high cell density and high bioproduct productivity. It is unique in creating a culture environment combining high oxygen transfer with low shear stress. Anticipated GMP production volumes will require a 500L-bioreactor and extensive process development to optimize yield, reduce cost of goods and identify critical process parameters will still need to be conducted to develop the at-scale GMP process. However, since the CelCradle uses the same TideMotion principle as the large-scale TideCell bioreactor, its small footprint and volume allows it to be used for process optimization and pipeline development.

In this Proof-of-Concept study, we have demonstrated that CelCradle-500AP can be used for the culture of Vero cells and the efficient production of JEV vaccine. The overall virus yield for one CC-500AP bottle was $1.06x10^{11}$ pfu (plaque assay); however, one CC-500AP bottle has a similar footprint to a T-175 flask but produces virus with a 21-fold higher productivity. Note that these high titres represent unoptimized culture conditions and subsequent experiments to determine the best operating parameters for Vero cell cultivation and JEV production will likely increase the yield even further.

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