WHITE PAPER
UPSTREAM BIOPROCESSING

Linear Scalability of CAR Lentivirus Production in Single-Use TideMotion® Bioreactors, from Laboratory to Industry Scale

Background and introduction

Single-use bioreactors were first introduced about a decade ago and has since become the benchmark for cGMP acceptance in the biomanufacturing industry. Many single-use bioreactors such as stirred-tank reactors (STRs), can only support suspension cell lines. In the current market, there are only a few bioreactors that can perform large scale culture of adherent cell lines, one of which includes VaccixCell's TideMotion® Bioreactors – CelCradle™ and TideCell®.

Chimeric Antigen Receptor T-cells (CAR-T) therapy is the latest new trend for cancer treatment. Given the rapid gain of interest, many groups are advancing their laboratory findings aggressively towards clinical phase with the need for large scale CAR-T production. One of the most common bioprocessing challenge remains in the inability to scaleup linearly. Whilst many parameters may be readily reproduced across scales, e.g., operating temperature and pH, others, such as dissolved oxygen transfer rate or shear stress, may not. Consequently, higher cost, effort and time will be required to re-optimize each scale-up. Such challenges and undesired conditions can easily be overcome by using TideMotion® bioreactors.

In this whitepaper, we focus on the linear scalability of CelCradle™ to TideCell® on transfection efficiency of HEK-293T using 4-plasmid lentiviral system to support large-scale LVV production.

Cells, Media and Materials

Hardware	CelCradle™ bioreactor
	TideCell® 2L matrix vessel
Cell Line	Adherent HEK-293T (ATCC: CRL-3216)
Growth Media	DMEM with High glucose (4.5 g/L); 10 % FBS 4 mM L-glutamine; 25 mM HEPES; 1X P/S
Growth Condition	37°C; 5% CO2; 75-85 % humidity;

Seed Generation Using Intensified Seed Train

To generate enough cells for LV production in large bioreactors, vials from a high-density cell bank were used for direct seeding into a single-use CelCradle™ bioreactor to generate adequate cell numbers needed for the LV production in TideCell®.

This intensified seed train strategy, as opposed to the conventional seed train, provides smaller working footprint, time-, cost- and manpower-effectiveness (Figure 1).

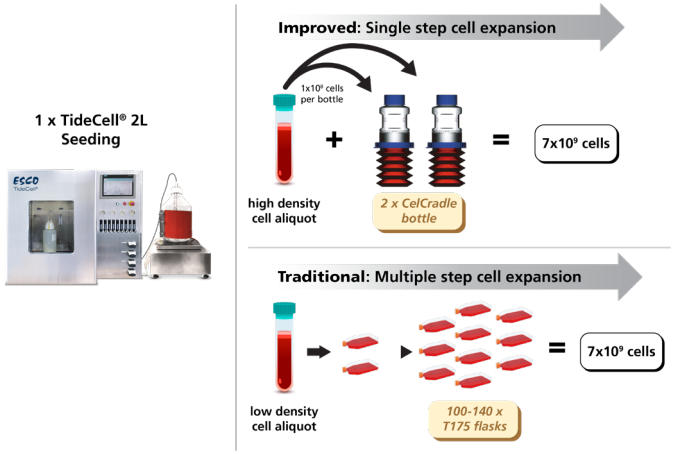

Figure 1: Compared to traditional methods of growing seed culture that is enough for performing 1 run of TideCell bioreactor, at least 100 to 140 plates of T-175 flasks are needed. This multiple-step cell expansion is extremely laborious. In comparison to using CelCradle™ and high-density seed train method, only a single step of cell expansion is required, which thereby reduces time, cost, effort and footprint drastically

Direct Translation of Protocol from CelCradle™ to TideCell® Platform Yields Linear Scaleup of LVV Production

Generally, HEK-293T cells are seeded on Day 0, transfected with 4-plasmid lentiviral system on Day 2 to induce LVV production. Viral supernatant will then be harvested every 24-hour for a total of three times (Figure 2).

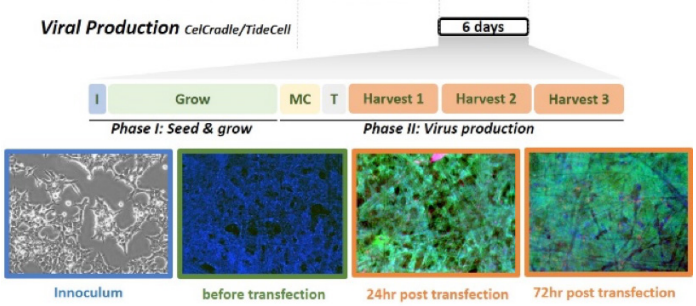

Figure 2: Overview of LVV production from seed culture to virus harvest takes approximately 2 weeks. Viral production takes 5 days including seeding, growing, transfection of viral system using PEI, and harvesting of virus supernatant.

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This exact process of LVV production was applied on both laboratory (CelCradle™) and manufacturing (TideCell®) scale. Infectious titer unit (TU) was determined using FACs with the help of a GFP reporter readout. Both scale of bioreactor demonstrates clear linear scalability with high viral titer of $1.0x10^9$ TU and $2.0x10^{10}$ TU in CelCradle™ and TideCell® 2L, respectively.

	CelCradle™	TideCell® 2L
Cell Expansion Vessel	5 x T175 flasks	2 x CelCradle™
BioNOC II Carriers	5.5 g	110 g
No. of Cells seeded	23,000 cells/cm^2	
Working volume	500 ml	10 liters
Attachment Time	180 min	
Attachment Efficiency	87 ± 7%	95% ± 4%
Transfection Timepoint	Day 2 post seeding	
No. of Cells (Day of Transfection)	27,000 cells/cm^2	
Transfection reagent	PEI "Max"	
DNA per Cell	4.2 x 10^{-12} g/cell	
Plasmid ratio	2:1:1:1 (pCDH: pMDL: pVSVG: pREV)	
Transfection hours	6-7 hours	
Harvest Timepoints	24h, 48h, 72h (3 harvests)	
Viral titer/cell	3 TU/cell	
Viral vector titer*	1.0 x 10^9 TU	2.0 x 10^{10} TU

Table 1: Linear scalability of LVV production between different scales of TideMotion bioreactors when protocol used on CelCradle were directly applied to TideCell platform.

TideMotion Bioreactors Prove to Have the Highest Overall Production Yield to Cost Ratio Compared to Alternative Platforms

When viral titer generated from CelCradle™ and TideCell® were compared to other culture platforms, Tide Motion bioreactors demonstrated to be the more efficient and cost saving platform.

	CelCradle™	TideCell® 2L
Viral Vector Titer*	1.0 x 10^9 TU	2.0 x 10^{10} TU
Equivalent to 10-cm dish (estimated ~$4x10^7$ TU)	25 x	500 x
Equivalent to Roller Bottle (estimated ~$4x10^8$ TU)	3 x	60 x
Equivalent to CF-10 (estimated ~$3x10^9$ TU)	0.3 x	6 x

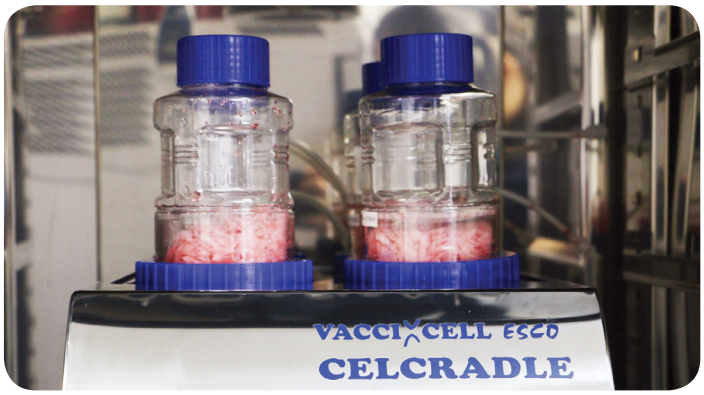
Table 2: Equivalent number of 2D vessels required in producing similar LVV yield compared to CelCradle™ and TideCell®.

	TideCell® 2L	10-cm dish	Roller bottle	Cell Factory 10
1X TideCell® 2L Equivalent	1X	500X	60X	6X
Manpower	+	+++++	+++	+
Hands-on Time Spent	+	+++++	+++++	+
Small Footprint	++	+++++	+++++	+
Close system capability	yes	no	no	no
Clean Room Needed	no	yes	yes	yes
Overall Cost-Effectiveness	+++++	+	+	+

Table 3: The use of Tide Motion® bioreactors are more cost-effective than other 2D platforms.

Summary

TideMotion® bioreactor is one of the leading single-used bioreactors that caters to the scaleup of adherent cell lines. Using novel high-intensity seed train, single-step cell expansion can be performed. With high oxygen transfer and low shear stress culture environment, our TideMotion technology ensures high cell mass and high productivity of target bioproducts. Users can easily collect highly concentrated cells, viruses or secreted products from TideMotion Bioreactor platforms. Most importantly, the ability to directly translate protocols from one scale to another at linear scalability, using our system, allow users to minimize optimization steps, saving precious time and effort.



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