CelCradle X® bioreactor-based scalable manufacturing of retrovirus-producing cells and retroviral particles for cost-effective CAR-T cell production

Lei Yang[1,^], Shijun Zha[2,^,#], Weiwen Luo[2,^], Sze-Wai Ng[1], Sean Jing-Xiang Tan[2], Arleen Sanny[1], Fong-Chan Choy[2], Xiangliang Lin[2,*], Kong-Peng Lam[3,4,5,6,7,*] & Andy Hee-Meng Tan[1,*]

[1]Immune Cell Manufacturing (ICM), Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), 20 Biopolis Way, #06-01 Centros, Singapore 138668, Singapore
[2]Esco Aster Pte. Ltd., 67 Ayer Rajah Crescent #02-01, Singapore 139950, Singapore
[3]Singapore Immunology Network, Agency for Science, Technology and Research, Singapore 138648, Singapore
Departments of [4]Physiology, [5]Microbiology and Immunology, and [6]Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117545, Singapore
[7]School of Biological Sciences, Nanyang Technological University, Singapore 637551, Singapore
[#]Presenting author ^These authors contributed equally
*Correspondence: andy_tan@bti.a-star.edu.sg, lam_kong_peng@immunol.a-star.edu.sg or xl.lin@escoaster.com

BACKGROUND

An increasing number of cell and gene therapy clinical trials are ongoing worldwide with fast-expanding pools of cancer antigen targets and therapeutic cell types. Among cell-based cancer therapies, chimeric antigen receptor (CAR)-T cell therapy has been growing profoundly with multiple FDA-approved products commercially available in the market. However, manufacturing of CAR-encoding viral vectors for T cell engineering constitutes a substantial proportion of the exorbitant cost of CAR-T therapies.

METHODOLOGY

To address this challenge, we developed a proprietary and scalable bioprocess based on a laboratory process to grow retrovirus-producing cells (VPCs) stably transduced with CAR to produce CAR retroviral particles (CAR RVPs) at clinical scale employing the CelCradle X® (CCX) platform. Process optimization resulted in a two-step approach to improve CAR vector transfection efficiency and production titer of CAR RV by a packaging cell line which enabled generation of stable CAR-expressing VPCs secreting CAR RVPs. RVPs were pseudotyped with an envelope glycoprotein different from what was used in the packaging step, so that they could endow a large fraction of T cells with CAR.

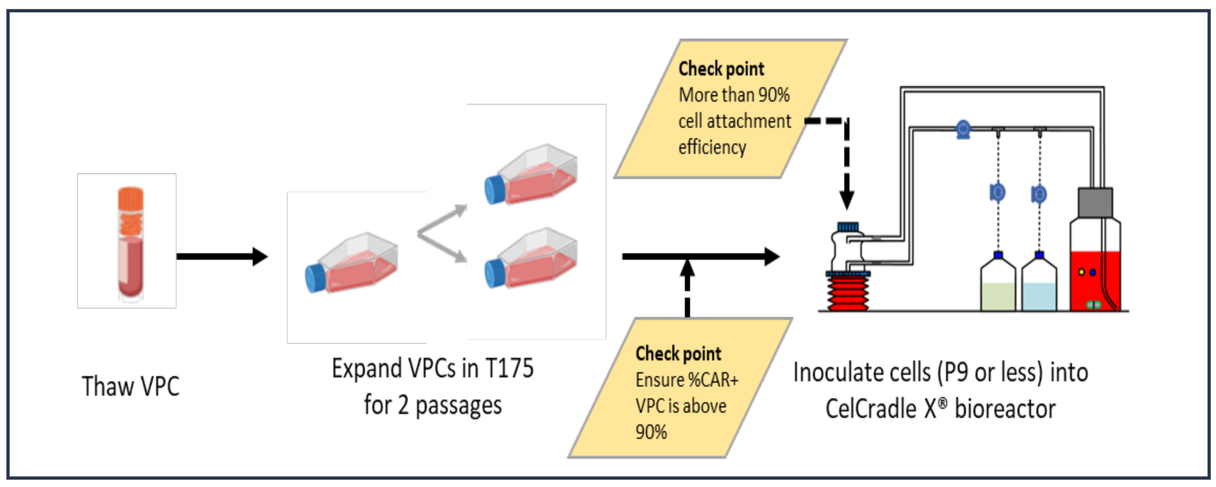


Figure 1. *Process overview of production of VPCs in the CCX bioreactor.*

RESULTS

We observed that CAR VPCs which underwent numerous passages of culture in the CCX bioreactor sustained high CAR expression, thus motivating the subsequent establishment of a VPC master cell bank (MCB) from which a working cell bank (WCB) was maintained to facilitate further work to enhance manufacturing robustness. For example, we optimized process parameters that supported increased culture scale of VPCs leading to high RVP yields.

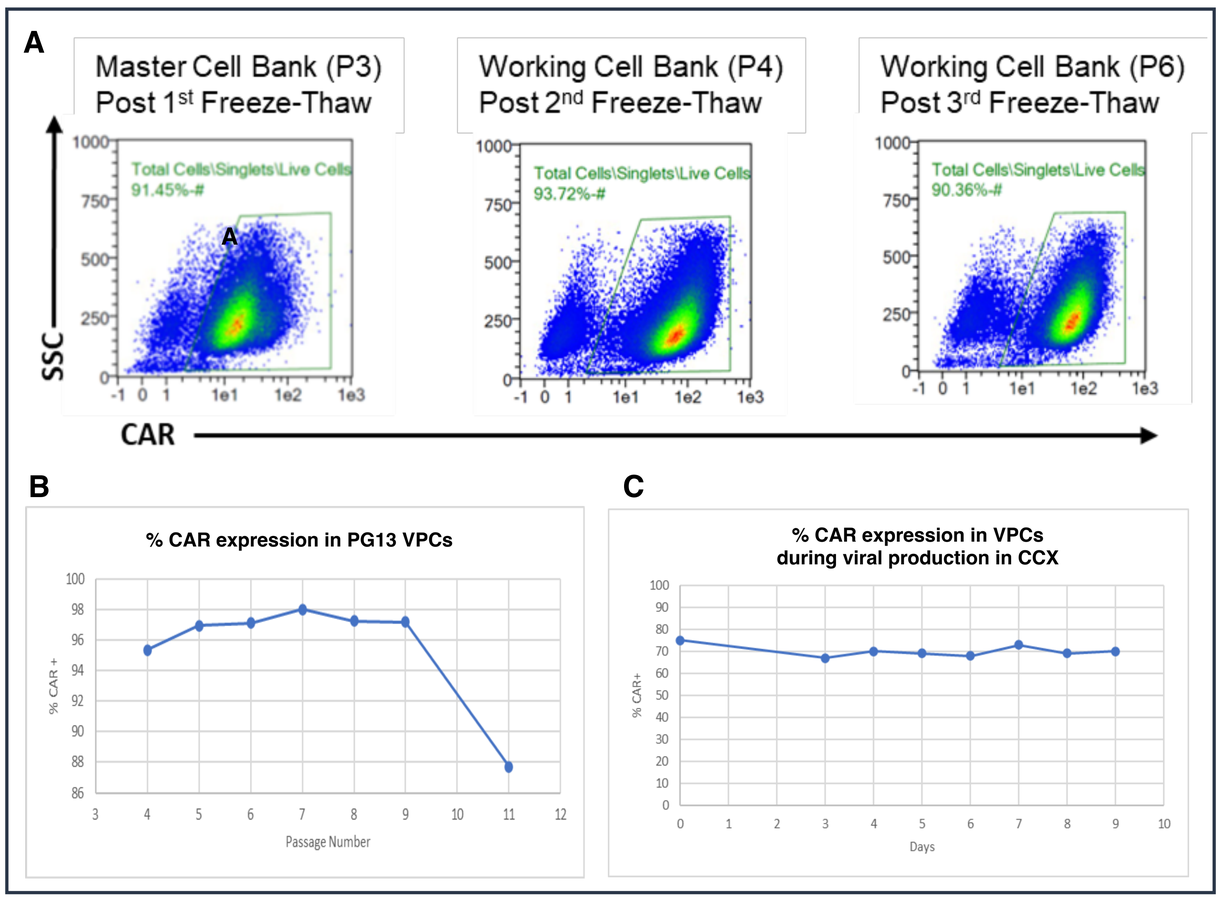


Figure 2. *Characterization of PG13 CAR VPCs.* ***(A)*** *Representative dot plots showing % CAR expression by VPCs from MCB and WCB as assessed by flow cytometry.* ***(B)*** *% CAR expression in VPCs at various cell passages.* ***(C)*** *Stable % CAR expression in PG13 VPCs during a 9-day culture process in the CCX bioreactor.*

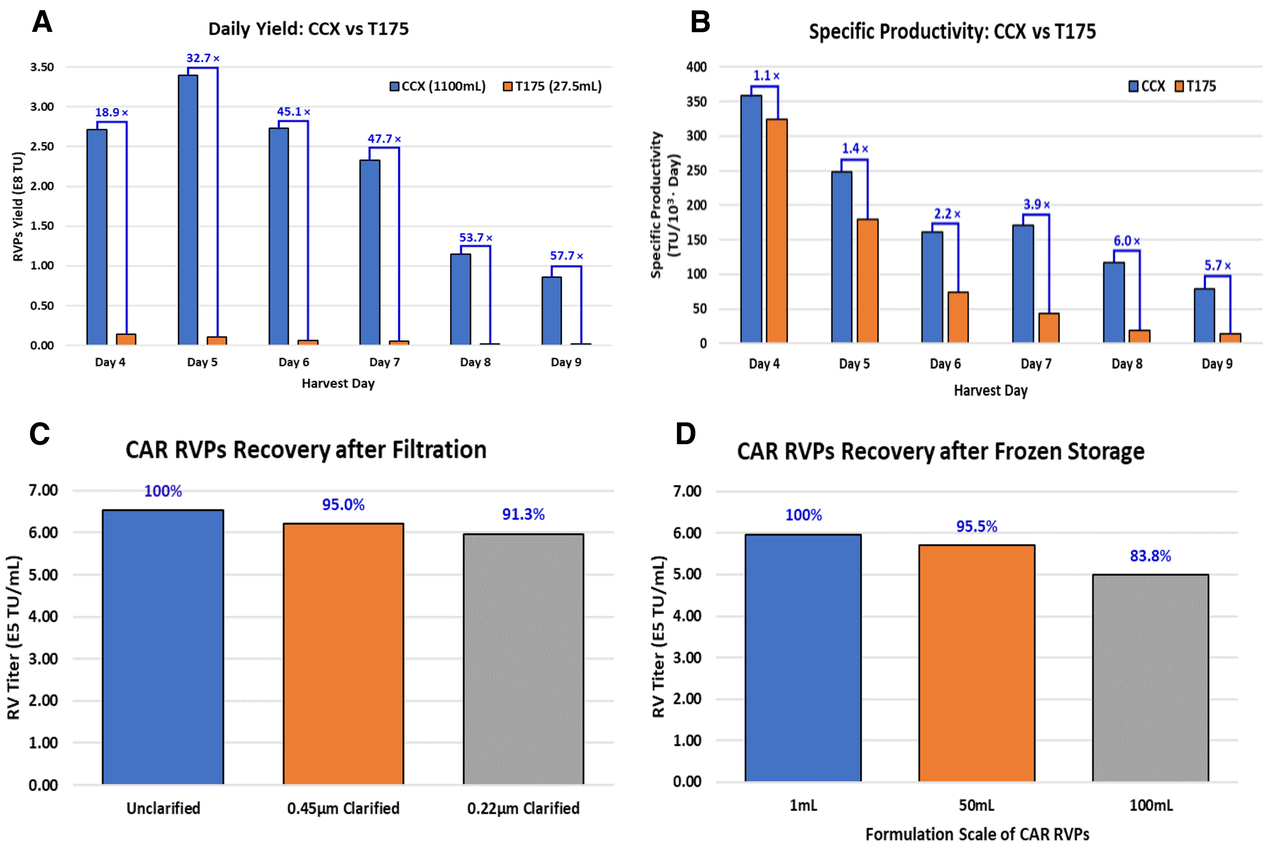
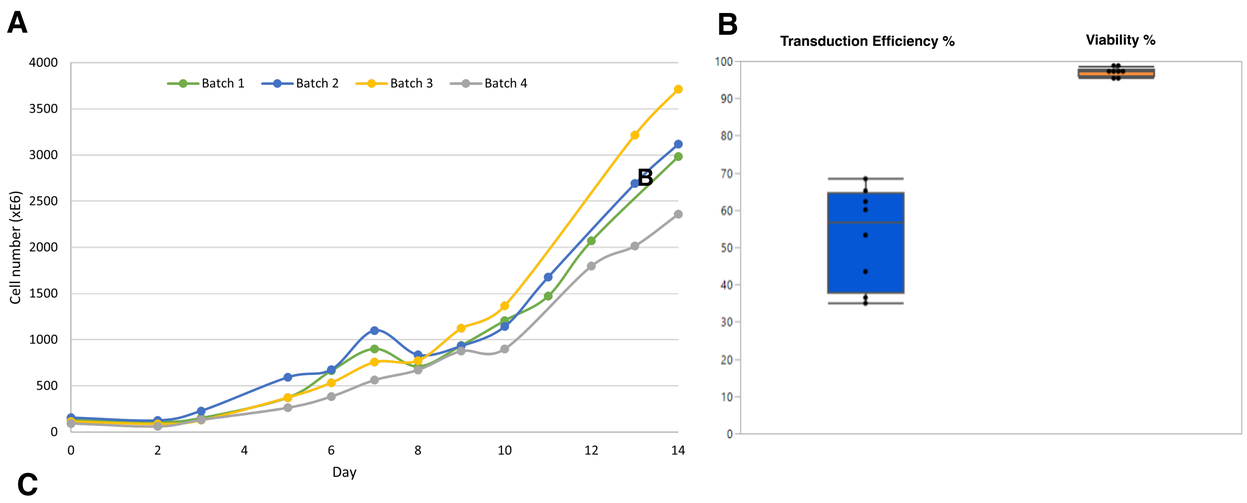


Figure 3. *Production of CAR RVPs by CCX bioreactor. Total yield* ***(A)*** *and specific productivity* ***(B)*** *of CAR RVPs harvested daily from CCX compared with T175. Recovery of CAR RVPs after filtration* ***(C)*** *and frozen storage* ***(D)***.

Importantly, CAR RVPs produced by our CCX system achieved consistently high efficiency of CAR transduction in T cells when expanded at a larger 1-to-3 L scale.



C

QUALITY ATTRIBUTES	UNIT	BATCH 1	BATCH 2	BATCH 3	BATCH 4
Donor	-	1803919001	1803919001	180682401C	190981104C
RV Lot	Run	1	2	3	4
RV Titer	× 10^5 TU/mL	2.0	3.0	3.0	5.6
Harvest Day	Day	11	13	10	13
CAR^+ T Cell Number	× 10^6 cells	515.38	1170.7	627.26	937.76
Transduction Efficiency	%	35.0	43.5	35.9	70.0
Viability	%	97.5	98.5	97.8	97.6
$CD3^+$	%	96.7	95.5	98.0	98.8
IFN-γ Release ($In\ CD8^+$ when culture with Nalm6)	%	16.85	15.89	49.36	16.45

Figure 4. *CAR-T manufacturing with CAR RVPs from CCX bioreactor.* ***(A)*** *Growth curves of CAR-T cells in different batches.* ***(B)*** *CAR transduction efficiency of T cells and viability of CAR-T cells generated using CAR RVPs from CCX bioreactor.* ***(C)*** *Quality attributes of CAR-T cells on the day of harvest.*

CONCLUSION

In conclusion, our study has developed a controllable and semi-closed CelCradle X® -based bioprocess which is also linearly scalable to produce CAR viral vectors with multiple harvests for clinical and commercial manufacturing of CAR-T cells, making CAR-T therapies more affordable.

ACKNOWLEDGEMENT

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