SEMI-AUTOMATED EXPANSION AND DOWNSTREAM PROCESSING OF MESENCHYMAL STROMAL CELLS FOR AUTOLOGOUS CELL THERAPY

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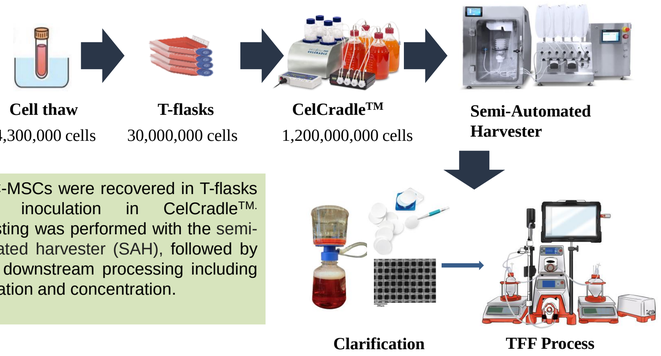
Introduction

In recent years, autologous cell therapies have received much attention in the field of regenerative medicine and potential treatment. Particularly, mesenchymal stromal cell (MSC) – derived therapies are considered the most mature cell-based therapies. It is expected that commercial bioreactors need to generate minimal batches of 10^8-10^9 cells as single-use batches for adequate production stability and operation efficiency. Esco Aster utilizes Tide Motion-based platforms for the expansion of anchorage-dependent cells to achieve sufficient therapeutic quantities of high-quality and well-characterised products. CelCradle™ bioreactor is integrated with automation modalities including perfusion-based feeding and real-time control of pH, as well as monitoring of dissolved oxygen (DO). This study will focus on the suitability and efficiency of the CelCradle™ along with its semi-automated harvester (SAH) for GMP autologous cell therapy application and the integration of up- and downstream processing workflow in compliance with GMP requirements.

A.



(A) UC-MSCs were recovered in T-flasks before inoculation in CelCradle™. Harvesting was performed with the semi-automated harvester (SAH), followed by 2-step downstream processing including clarification and concentration.

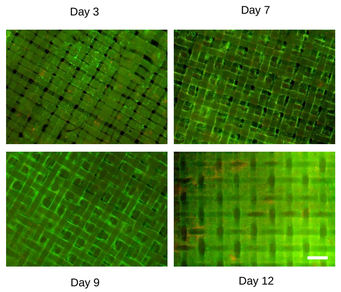
CelCradle™ Growth and Expansion

(B) Fluorescent stain images displayed the typical expansion of UC-MSCs on BioMesh™ carriers in a Celcradle™ over 13 days. Green: Fluorescein diacetate (live cell cytoplasm); Red: propidium iodide (dead cell nuclei). Scale bar: 200 µm

(C) UC-MSCs were seeded into CelCradle™ at 45,000 cells per carrier to achieve 2,100,000 cells per carrier with a fold expansion of 46.89 (5.32 doublings) over 13 days of culture.

(D&E) Real time monitoring of pH (D) and DO (E).

B.



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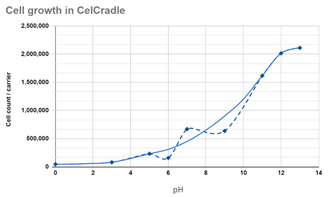
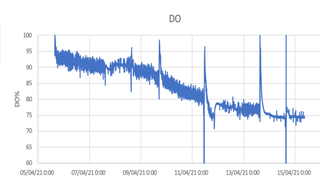


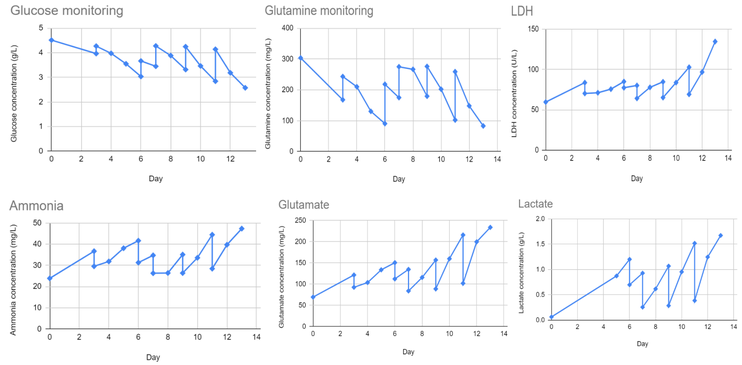
D.

E.



Metabolites Monitoring

F.



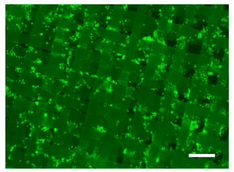
(F) A bioanalyzer was employed for daily monitoring of metabolism throughout the culture period. Measured values of glucose, glutamine, and other waste-products presented in the media were within accepted ranges for cell growth, indicating adequate media change frequency.

Cell harvesting by Semi-automated Harvester

(G) Fluorescent staining indicates efficient harvesting with the semi-automated harvester. Green: Fluorescein diacetate (live cell cytoplasm). Scale bar: 200 µm

(H) The extrapolated cell count of cells harvested from 3 random carriers from the CelCradle™ provides an estimate of the total cell count in the entire packed bed. Mass harvesting efficiency of the entire packed bed is 94.29%, while assuming estimated total cell count from small scale sampling as 100%.

G.



H.

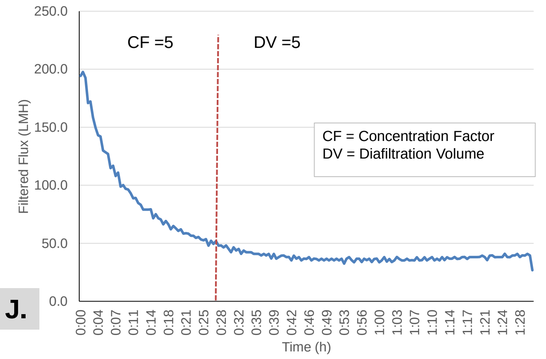
	Estimated from 3 Carriers (x)	Semi-Automated Harvester (SAH)	
		Harvested by SAH (y)	Harvesting Efficiency (y/x * 100)
Total Live Cells	1,276,550,000	1,203,616,071	94.29%
Viability (%)	95.50	94.09	

Downstream processing

I.

	Time spent
Clarification	20 min
5x Concentration	27 min
5x Diafiltration	1 hr 04 min
Total Duration	2 hr

J.



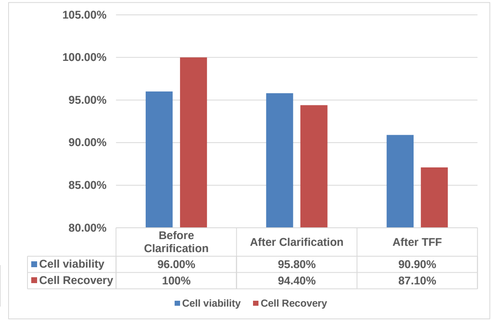
(I) The entire downstream process lasted 2 hours. Clarification was conducted by filtering thrice with nylon mesh, followed by tangential flow filtration (TFF) for purification of the MSCs.

(J) Approximately 30 minutes was required for 5x volume reduction, followed by an additional 30 mins for 5x diafiltration. The average filtered flux during the diafiltration phase was around 50 LMH, shortening TFF process time.

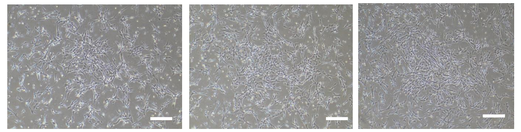
(K) Cell recovery efficiency and cell viability after each step of downstream process. After TFF processing, more than 80% of cells were recovered with viability over 90%.

(L) Cells were re-seeded in 12-well plates after each step and demonstrated normal re-attachment abilities plate normal morphology. Scale bar: 200 µm

K.



L.



Conclusion

- **Overall, UC-MSCs were expanded from 4,300,000 to 1,203,616,071 over 17 days, equating to a 279,91-fold expansion, or 8.13 population doublings, whilst maintaining a viability of 94.09%.**
- **Downstream clarification and TFF process required only 2 hours with 87.10% cell recovery achieved with cells maintaining over 90% viability.**
- **This study indicates the suitability of Tide Motion bioreactors in the culturing of mesenchymal stromal cells. Particularly, the integration of the upstream CelCradle™ together with the Semi-Automated Harvester and downstream TFF as a viable up- and downstream process for GMP autologous cell therapy applications and allogenic seed cells preparation.**

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