LARGE SCALE 3D BIOREACTOR TECHNOLOGY: LINEAR SCALE UP OF MESENCHYMAL STROMAL CELL EXPANSION WITH TIDEXCELL™ SYSTEM

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Introduction

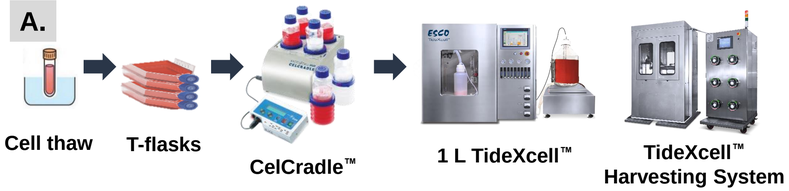
Mesenchymal stromal cell (MSC) therapy requirements can range from several to over 300 million cells per dosage. Conventional expansion of MSCs on plasticware is impractical when larger dosages of over 50 million cells are required. The use of bioreactors combining scaling-up ability, process control and automation is an effective solution to overcome this difficulty, however, many bioreactors face issues in supporting MSC cultures due to complications in balancing the need for adequate mixing of media whilst retaining minimal shear stress, and achieving the ability to separate cells from micro/macro carriers with both high cell yield and viability.

Tide Motion bioreactors are a robust and scalable microcarrier platform to meet the rigorous demands of clinical therapies, working in tandem with the TideXcell™ Harvesting System (TXLHS) to provide automated, high efficiency packed bed cell harvesting.

Bone marrow (BM) derived MSCs were seeded and expanded within PET macrocarriers, BioNOC™ II, several times via seed train with parameters such as glucose consumption and pH levels measured to ensure proper culture scale-up. In addition, harvesting of the 1 L packed bed was tested with the TXLHS. Overall, we present our process optimization with quality controls and release criteria of functional and phenotypic characteristics for the translation of academic/industrial R&D into bench sale for future clinical trials and commercialization processes.

Autologous production → **Allogeneic production**

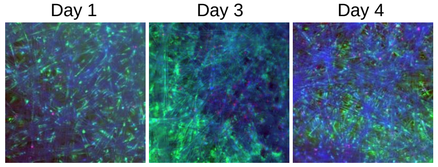
A.



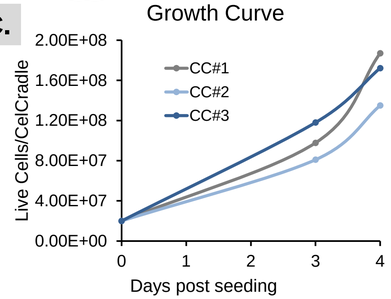
(A) BM-MSCs were recovered in T-flasks, detached and expanded via seed train: seeding, expansion and harvesting in the CelCradle™, a small benchtop bioreactor, followed by seeding and expansion in the TideXcell™ bioreactor containing 1 L packed bed volume and harvesting performed with the TideXcell™ Harvesting System.

CelCradle™ Growth and Expansion

B.



C.

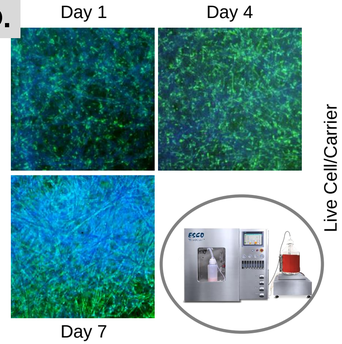


(B) Fluorescent images display the typical expansion of BM-MSCs on BioNOC™ II carriers in a CelCradle™ over 4 days. Green: Fluorescein diacetate (live cell cytoplasm); Blue: Hoechst 33342 (nuclei); Red: propidium iodide (dead cell nuclei).

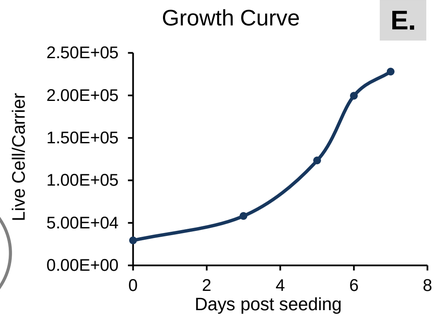
(C) BM-MSCs expanded an average of 2.95 doublings in the Celcradle™ over 4 days

TideXcell™ Growth and Harvesting

D.



E.



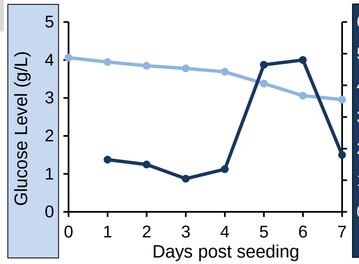
(D) Fluorescent images display the expansion of BM-MSCs on BioNOC™ II carriers in the TideXcell™ over 7 days, which is comparable to the observed growth in the CelCradle™. Green: Fluorescein diacetate (live cell cytoplasm); Blue: Hoechst 33342 (nuclei)

(E) The characteristic cell growth curve consisting of the lag, exponential and plateau phases is still observed on the 1 L packed bed of BioNOC™ II carriers in the TideXcell™.

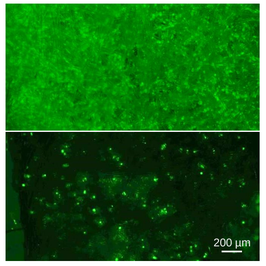
(F) Glucose consumption rate in the TideXcell™ is proportional to the growth rate of cells. Ample glucose was available throughout the culture period, avoiding the need for media replacement via the use of the TideXcell™ system from day 0 of culture.

(G) Fluorescent before (top) and after (bottom) images indicate efficient cell harvesting with the TideXcell™ Harvesting System. Green: Fluorescein diacetate (live cell cytoplasm).

F.



G.



H.

	Estimate from 10 Carriers (x)	TXLHS Harvested Cells		
		Before centrifugation (y)	After centrifugation	Harvesting Efficiency (y/x * 100)
Total Live Cells	1,938,000,000	1,892,800,000	1,615,040,000	97.67%
Viability	92.68%	93.81%	95.20%	

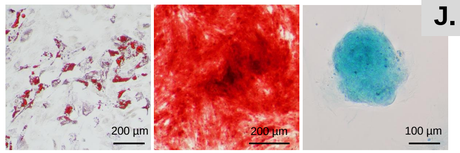
(H) The extrapolated cell count of cells harvested from 10 random carriers from the TideXcell™ provides an accurate estimation of the total cell count of the entire 1 L packed bed. Centrifugation, was found to reduce the obtained cell amount by 14.7%. Both high harvesting efficiency and viability were achieved with the TXLHS with ease.

Post Harvest Cell Quality Indicators

I.

Surface Markers	% of BM-MSC
CD73+	99.81
CD90+	99.98
CD105+	98.65
CD34- CD11b- CD19- CD45- HLA-DR-	99.91
CD90+ CD105+ CD73+ CD34- CD11b- CD19- CD45- HLA-DR-	98.50

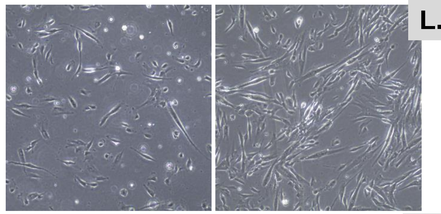
J.



K.

IFN-γ	50 U/ml	200 U/ml	800 U/ml
IDO%	14.4	26.8	33.4
IPBv of IDO	240.2	306.8	357.0

L.



(I) BM-MSCs retained > 98% for all positive and < 2% for all negative MSC surface marker criteria as established by the international society of cell and gene therapy (ISCT).

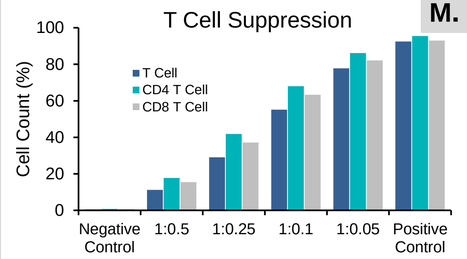
(J) Trilineage differentiation capability was observed to be present in harvested cells. Cells were induced to differentiate for 28 days.

(K) BM-MSCs frozen after TXLHS harvest were recovered, stimulated with IFN-γ and measured for IDO expression.

(L) Frozen BM-MSCs retained plastic adherence with 92.3% viability and continued to expand when recovered in T-175 flasks. Day 1 of recovery (left), day 3 (right).

(M) BM-MSCs frozen after TXLHS harvest were recovered, cultured together with T cells and the resulting T cell growth suppression was recorded. The average T cell suppression rate was found to be 53.2%.

M.



Conclusion

+ Tide Motion bioreactors together with the TXLHS serve as an efficient, linearly scalable, one-stop BM-MSC expansion and harvesting platform with flexible cell yields depending on variable packed bed volumes and back-to-back seed train expansions.

+ Small scale systems (CelCradle™) support early developmental stage testing, allowing for optimization of parameters at low costs before scaling up with the larger TideXcell™.

Resources:

Cultivation and Expansion of Human Mesenchymal Stem Cells in Tide Motion Bioreactor

Optimizing Large-Scale Expansion of Mesenchymal Stem Cells in 3D Tide Motion Bioreactors

Protocol for Culturing MSCs in CelCradle™

Find out more at http://www.vaccixcell.com or http://www.escoaster.com
For enquiries, contact Esco Vaccixcell (Bioprocessing Tools): mail@vaccixcell.com or Esco Aster (cGMP CDMO): mail@escoaster.com

Seed Train	Population Doublings	Days	Doubling Time (h)	Number of cells after harvest (M)
T-flask	3.73	4	25.7	33.2
CelCradle™	2.95	4	32.6	250.0
1 L TideXcell™	2.92	7	59.9	1800.0
Overall	9.60	15	37.9	1800.0

Tide Motion bioreactor	Matrix volume (L)	Seeding count (Cells)	Expected harvest* (Cells)	Number of doses**
CelCradle™	0.1	1 - $3e^7$	1.8 - $8e^8$	3 - 12
TideXcell™	2	2 - $6e^8$	3.6 - $16e^9$	57 - 256
	20	2 - $6e^9$	3.6 - $16e^{10}$	576 - 2560
	100	1 - $3e^{10}$	1.8 - $8e^{11}$	2880 - 12800
	300	3 - $9e^{10}$	5.4 - $24e^{11}$	8640 - 38400

* Expected harvest performed at 90 - 100% confluency. This number varies with different harvesting methods, media type used, cell age and MSC type.

** Assuming each dose requires $5e^7$ cells, with an 80 % recovery rate after both post harvest downstream processing and cryopreservation loss.