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

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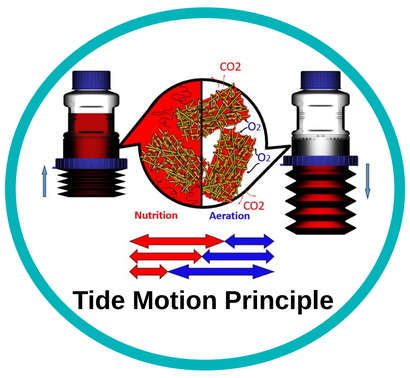
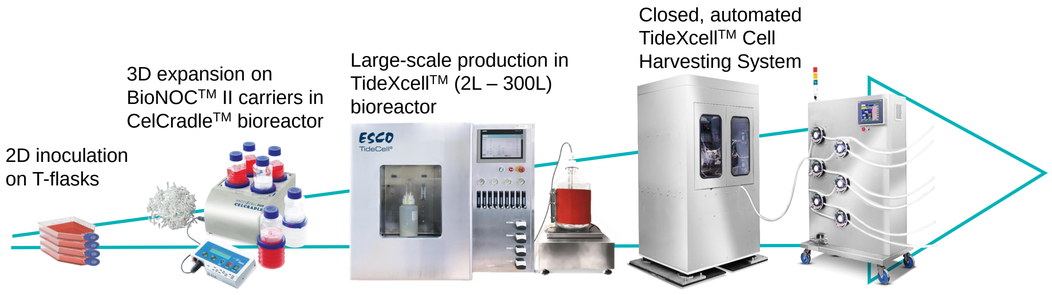
Introduction

The future cell therapy demands high quantities of Mesenchymal stem cells (MSCs) ranging from 10 million to more than 200 million cells per dosage. Conventional expansion of MSCs on plastic wares become impractical when large dosages of more than 50 million cells are required. The use of bioreactors which combines scaling-up ability, process control and automation is the primary solution for this need. Many bioreactors are facing issues in supporting MSC cultures due to complications in balancing the need for proper mixing of media with the need to minimize shear stress as well as the inability to separate cells from micro/macro carriers with high cell yield and viability.

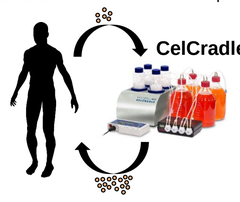
Esco Aster has leveraged on the Tide Motion bioreactors to establish a robust and scalable platform using macrocarriers to meet the demands for future clinical therapies. MSCs isolated from different tissues sources were seeded and allowed to expand within PET macrocarriers. Throughout culture periods, cell culture conditions were monitored, with bioprocess parameters such as glucose consumption and pH levels measured to ensure proper scale-up. Key issues such as cell seeding densities, media culturing conditions and improved bioprocess parameters needed for optimal stem cell systems were studied in our system. 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 process.



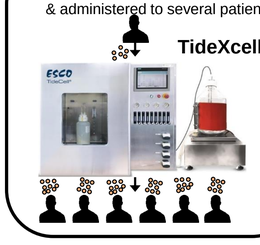
Tide Motion Principle



Autologous (to self)
Patient-derived cells expanded in CelCradle™ & infused back into patient

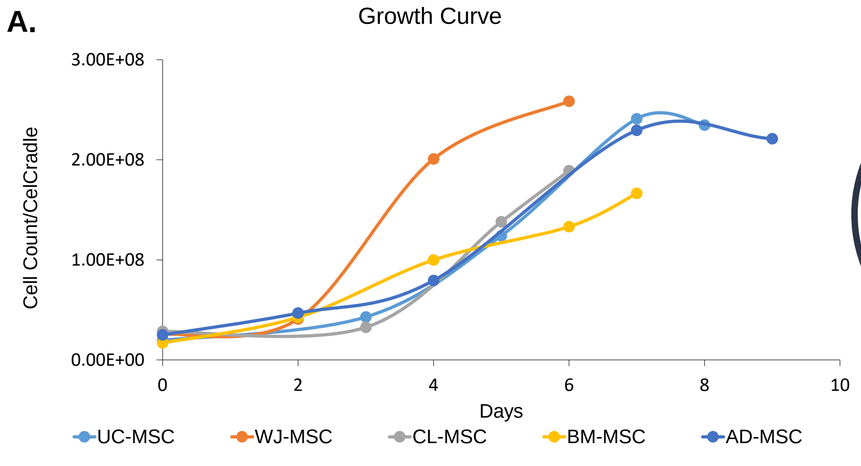


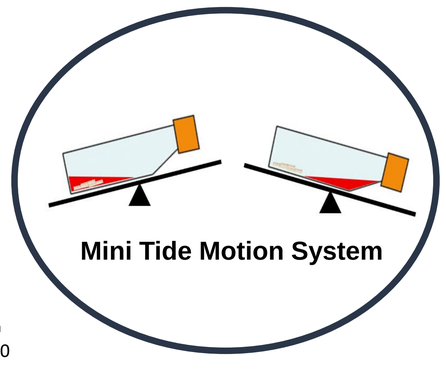
Allogenic (one to many)
Donor cells expanded in TideXCell™ & administered to several patients



Celcradle™ and TideXcell™ bioreactors can be integrated with cell processing isolators

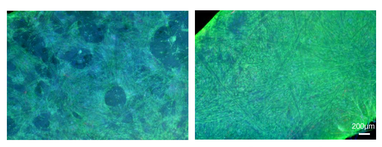
Growth of MSCs of Different Tissue Origins





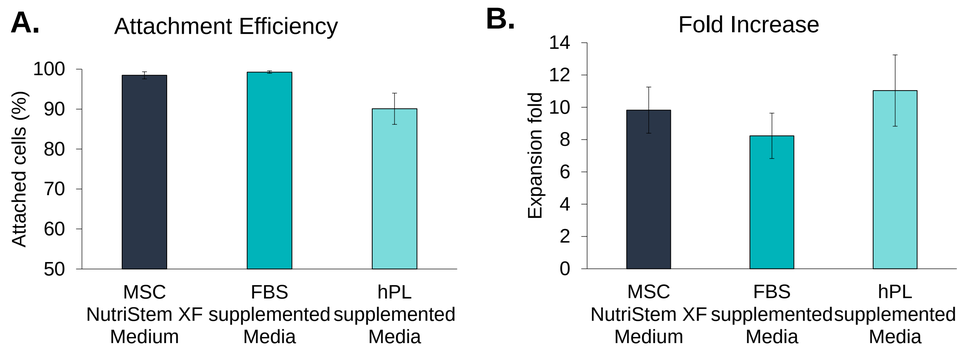
Tissue Origin	Cells Seeded	Cells Harvested	Fold Increase	Culture Days
UC-MSC	1.96E+07	2.41E+08	12.33	7
WJ-MSC	2.55E+07	2.59E+08	10.14	6
CL-MSC	2.85E+07	1.89E+08	6.64	6
BM-MSC	1.70E+07	1.66E+08	9.79	7
AD-MSC	2.50E+07	2.30E+08	9.15	9

B.

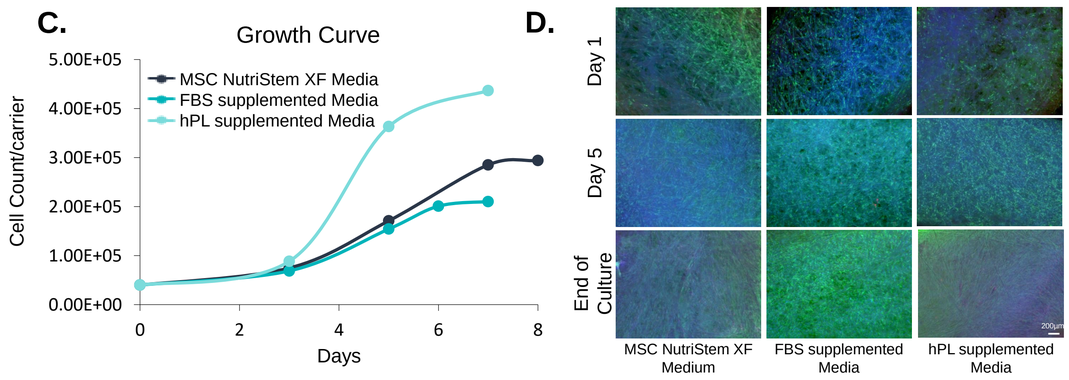


(A) Successful expansion of MSCs from different tissue origins have been demonstrated in the system. Common tissue origins of MSCs such as umbilical cord-derived MSC (UC-MSC), Wharton Jelly-derived MSC (WJ-MSC), bone marrow-derived MSC (BM-MSC) and adipose-derived MSC (AD-MSC) generally show a 10-fold expansion within 7 days of culture. The table above lists the recommended seeding density for the various tissue sources of MSC optimized using commercial sources of the cells. (B) Different sources of MSCs from the same tissue origin were tested for their ability to expand from a low seeding density. Florescence imaging of the cells on the carriers at the end of the culture period show successful expansion to a confluent level by BM-MSCs obtained from source 2 unlike MSCs from source 1. As differences in growth parameters were observed between MSCs from different sources, optimization of parameters such as seeding density and media change regime is usually conducted for each source of MSC prior to expansion in bioreactors of a larger scale.

Expansion of MSCs in Different Media Types



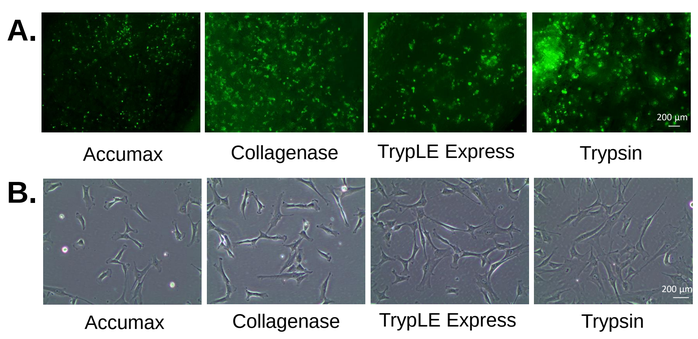
(A) Lower attachment efficiency on the carriers was observed when UC-MSCs were seeded in hPL supplemented media. (B) Despite the lower attachment efficiency observed, hPL supplemented media gave rise to the greatest expansion of cells across MSCs from different tissue origins.



(C) UC-MSCs were adapted to the different media types over 2 passages before seeding onto BioNOC II carriers for monitoring of proliferation rates. (D) Fluorescent images show the expansion of the cells on the carriers over the culture period. Green: Fluorescein diacetate (cytoplasm of live cells), Blue: Hoechst 33342 (nucleus), Red: propidium iodide (dead cells)

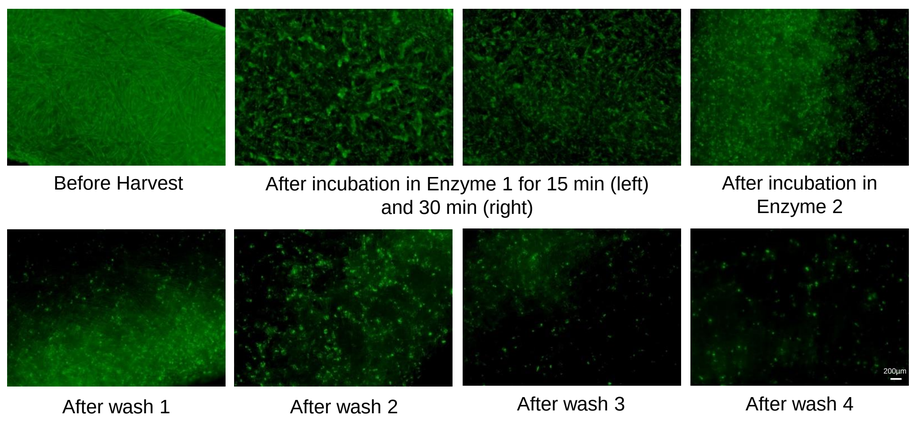
Optimization of Harvesting Process

Testing of Different Enzymes

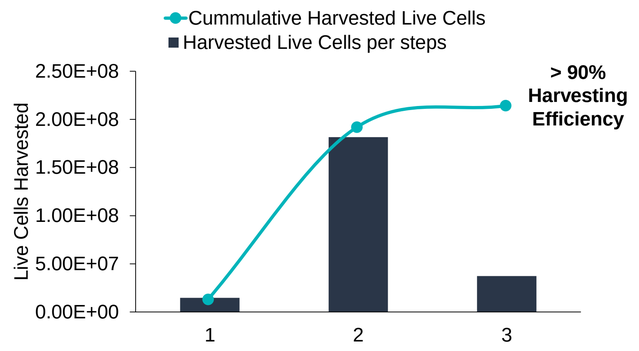


	Accumax	Collagenase	TryPLE Express	Trypsin
Harvesting (%)	87	68.3	78.3	56.3
Viability (%)	95.6	73.2	95.8	88.9

Mass Harvesting from CelCradle™



(C) Through various efforts at optimizing harvesting parameters, a process was developed for efficient mass harvesting of UC-MSCs from BioNOC II carriers. Fluorescein diacetate (FDA) was used to stain for remaining live cells on BioNOC II carriers were sampled throughout the harvesting process.



Harvest	Live Cells Harvested	Viability	Total Live Cells Harvested
Enzyme 1	14,625,000	94.35%	233,375,000
Enzyme 2 + Wash 1	181,500,000	96.80%	
Wash 2 – 5	37,250,000	95.82%	

Quality Checks

Classification	Parameters	Procedure	Reference value
Quantity	Cell Count	Automated cell counter	Depending on dose per vial (Range from 10 million to 1 billion)
Viability	Cell viability	Automated cell counter	>90%
Identity	Karotype	Cytogeneticists study	Normal Karyotype
Identity	Morphology	Visual microscopy observations	Fibroblastic spindle shape
Identity	Trilineage differentiation	Differentiation assay (Adipogenic, Chrondrogenic, Osteogenic)	Pos
Identity/ Purity	Stemness	Immune phenotyping by FACS	≥ 95% CD105, CD73, CD90 ≤ 2% CD34, 45, HLA DR, CD11b, CD19
Safety	Sterility <USP71>	Automated microbial detection system	Neg
Safety	Mycoplasma <USP63>	PCR	Neg
Safety	Endotoxin <USP85>	Limulus Amebocyte Lysate	<0.25EU/kg
Safety	Viral (HIV, HBV, HCV, HPV)	Viral protein antigen (ELISA)	Neg
Safety	Tumorigenicity tests	Telomerase enzyme activity	RTA<1.2
Potency	Cytokine protein expression assay	eg IL-6 protein secretion (ELISA)	
Potency	Cytokine gene expression assay	eg TNF-α gene expression (q-PCR)	
Potency	MSC migration/adhesion	Scratch test/ Transwell assay	
Potency	Immunomodulatory assay	IDO expression and activity assay	

Conclusion

- Tide Motion bioreactors consists of a series of single-use, loose packed-bed bioreactors of increasing scale.
- They serve as a platform for high capacity manufacturing of MSCs in single batches.
- Small scale systems support early developmental stages of the bioconversion process, allowing for optimization of parameters at low costs.

Factor/Attribute	Performance of MSCs
Cell Types	Suitable for various tissue origins of MSCs (UC, WJ, BM and AD)*
Cell Attachment	High seeding efficiency >90% Fibronectin coating for serum free media (suitable for cGMP production)
Cell Growth & Monitoring	Ease of visualizing cells on matrix via dye stain Confluence achieved at day 6-8 with ~10X expansion
Cell Harvest and Media Collection	>90% cells harvested with suitable enzymes Permits collection of media for processing of exosomes or conditioned media
Quality of cells	Stemness and trilineage differentiation of MSCs preserved Viability of harvest >90%, healthy cells obtained after harvest

Current seeding at 2e7 cells per CelCradle™ to obtain 2e8 cells
Projected to obtain 4e9 cells in 2L TideXcell™ and 6e11 cells in 300L TideXcell™**

*Growth parameters of other tissue origins of MSCs scheduled for development
**Final density will vary based on age, source of stem cells and media type used

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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