CULTIVATION AND EXPANSION OF HUMAN MESENCHYMAL STEM CELLS IN TIDE MOTION BIOREACTORS

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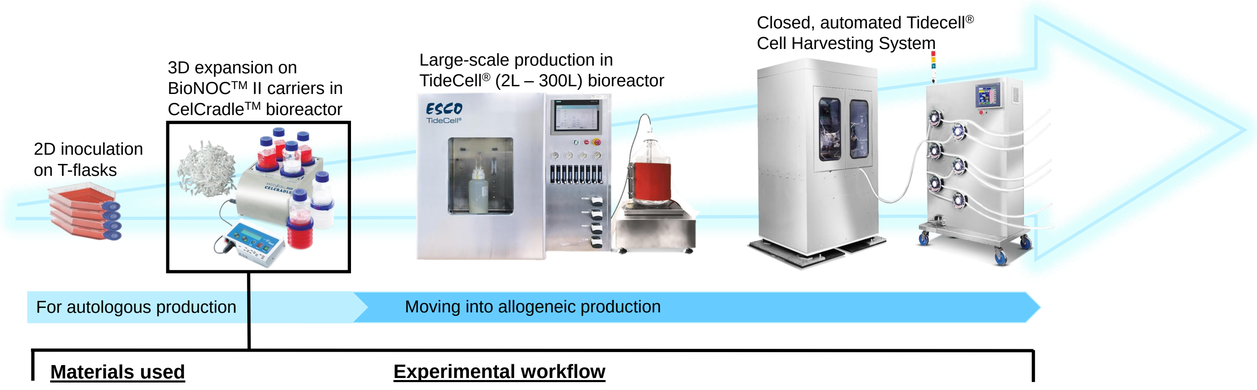
INTRODUCTION

Human mesenchymal stem cells (MSCs) have received great medical interest as a new treatment option and spurred a new age in regenerative medicine. However, conventional cultivation on plasticwares and in suspensions are difficult to scale-up production of MSCs for clinical applications. To overcome these challenges, Esco Aster has leveraged on Tide Motion bioreactors to develop a scalable bioprocess operation for the production of MCSs in a Current Good Manufacturing Practices (cGMP) compliant process. Human MSCs isolated from healthy donors were expanded in conventional 2D adherent cultures for a few passages before seeding into macrocarriers (BioNOC™ II) in a CelCradle™ bottle. The cells were grown in chemically defined media and harvesting efficiencies of more than 90% were achieved, with cell viabilities greater than 85% after 5-7 days in culture. In accordance to International Society for Cellular Therapy (ISCT), quality control and release criteria for MSCs characterized by their surface markers and multipotency (adipogenic, osteogenic and chondrogenic differentiations) ensured more than 95% of MSCs in culture. Importantly, MSCs cultured on the BioNOC™ II displayed similar *in vivo* characteristics, with secretions of extracellular matrix (ECM) proteins and fibroblastic morphological changes. Our current process is robust, relying on standard bioprocessing tools in most contract manufacturing facilities (CMOs). Through monitoring and optimization of key process parameters, such as pH, glucose consumption rates, we aim to easily translate lab scale production from academic/industrial R&D into bench scale/ pilot scale of clinical trials and commercial production.

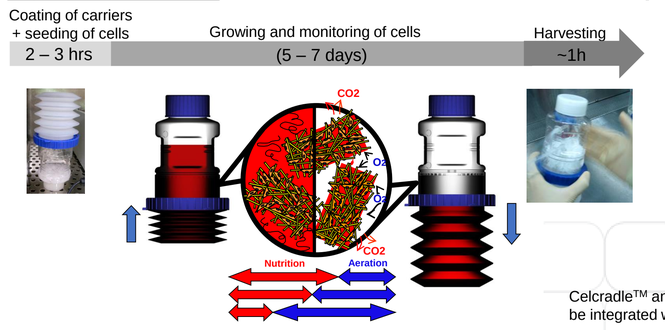
PROCESS DEVELOPMENT



Materials used

- P6 Cord-Lining MSCs (CL-MSC) generously provided by CellResearch Corp, Singapore
- MSC NutriStem® XF Basal Medium supplemented with MSC NutriStem® XF Supplement Mix (Biological Industries, Israel)
- CelCradle™ 500A (VacciXcell, Singapore)

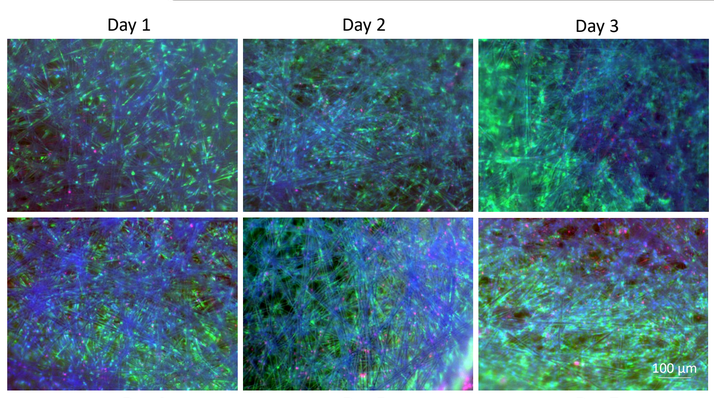
Experimental workflow

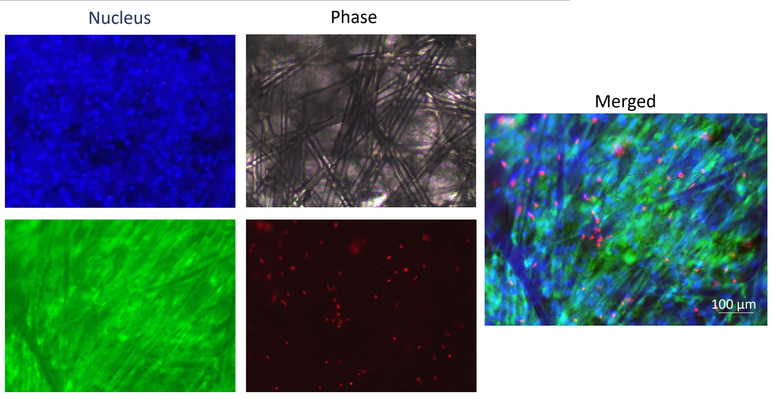


Celcradle™ and Tidecell® bioreactors can be integrated with cell processing isolators

RESULTS

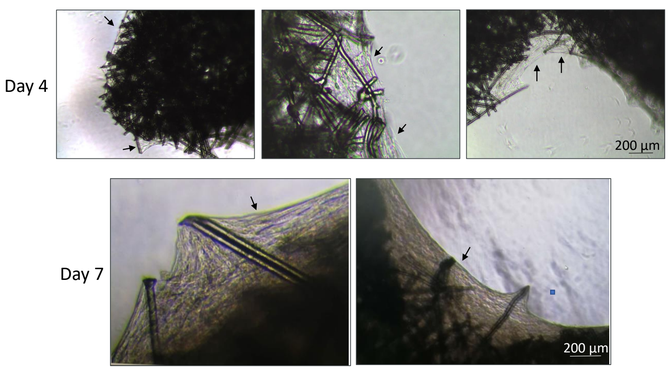
MONITORING MSCs EXPANSION IN CELCRADLE™





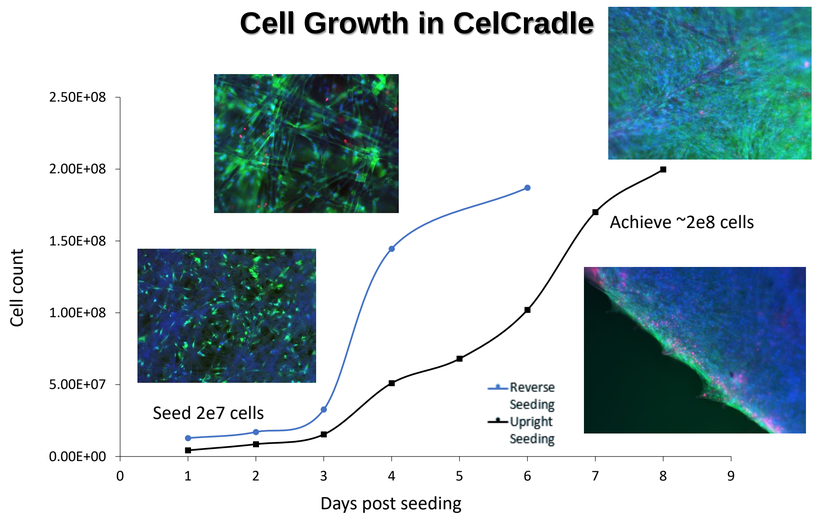
Green: Fluorescein diacetate (cytoplasm – live cells), Blue: Hoechst 33342 (nucleus – all cells), Red: propidium iodide (nucleus - dead cells)

High MSCs Confluence in BioNOC™ II

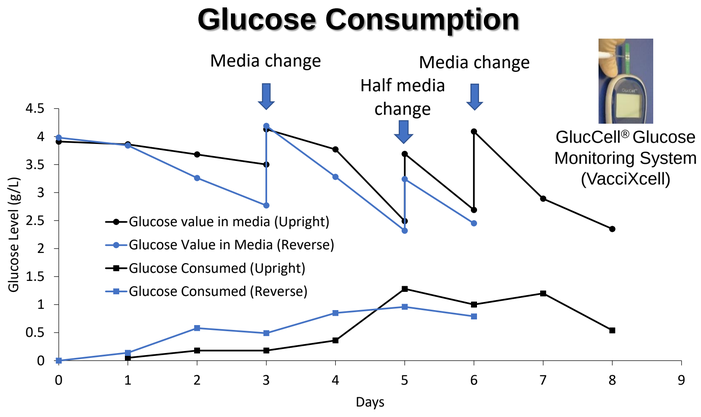


Cells can be observed protruding from edge when at high confluence – an indicator of cells achieving max growth

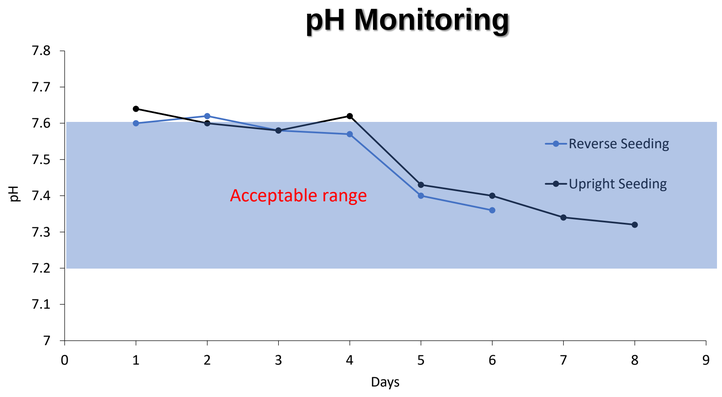
Cell Growth in CelCradle



Glucose Consumption

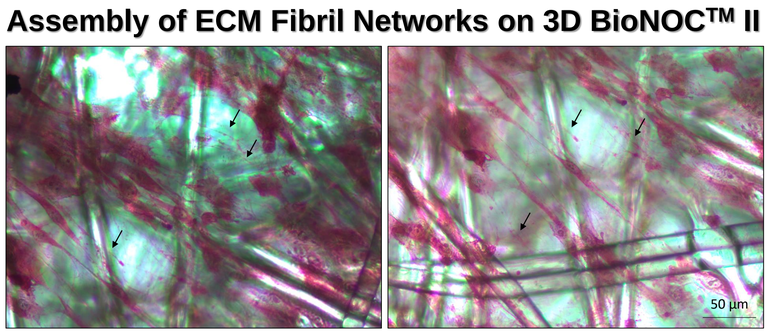


pH Monitoring

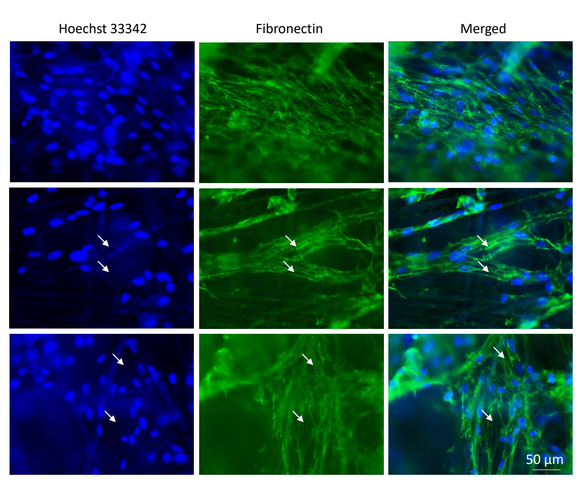


ECM FORMATION

Assembly of ECM Fibril Networks on 3D BioNOC™ II



Black arrow points to ECM in CL-MSCs cultured for 4 days in BioNOC™ II – staining of collagen fibres with picro sirius red



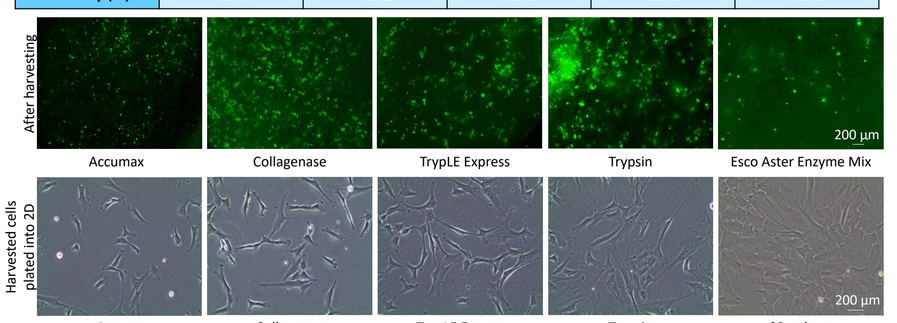
ECM-Fibronectin secreted by MSCs

Network alignment of fibronectin observed in MSCs – cells appear in unidirectional alignment

White arrows point to fibrils of extracellular matrix (ECM) fibronectin

CELL HARVESTING

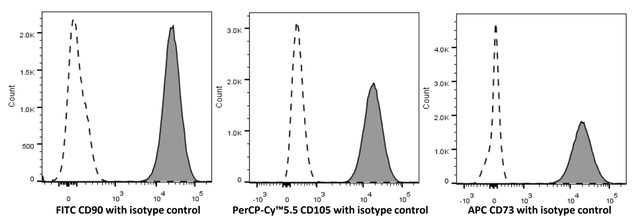
Enzymes	Accumax™	Collagenase	TrypLE™ Express	Trypsin	Esco Aster Enzyme Mix
Harvesting (%)	87	68.3	78.3	56.3	91.8
Viability (%)	95.6	73.2	95.8	88.9	93.5



*Harvesting efficiencies varies for different stem cell type and cocktail of enzymes used

QUALITY CONTROL & RELEASE CRITERIA

Cell Identity

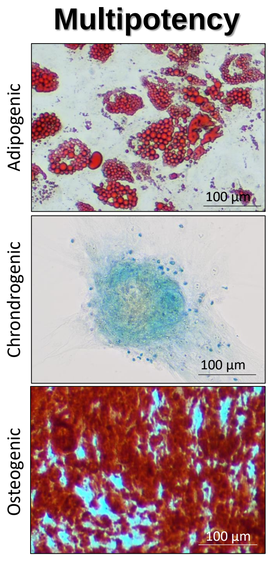


CL-MSCs harvested from BioNOC II at P7

Name	Statistic	#Cells
Specimen_001_sample_001.fcs		100000
P1	66.2	66207
CD73+ CD90+	99.6	65923
CD73+ CD105+	99.8	65789
CD34- CD45- HLA-DR-	98.5	64787

98.5% of MSCs retain stemness in 3D BioNOC II

Multipotency



CONCLUSIONS

- Single-use fixed-bed bioreactor, with ease of translation from bench to industrial scale
- Simplified seed train operations
- Expansion of MCSs in a Current Good Manufacturing Practices (cGMP) compliant process

Factor/Attribute	Performance of MSCs
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 5-8 Control and monitoring of process parameters
Cell Harvest	>90% cells harvested with suitable Esco Aster proprietary enzymes
ECM Secretion	Fibronectin and collagen observed More relevant to *in vivo* conditions with 3D growth
Quality of Cells	Stemness and trilineage differentiation of MSCs preserved Viability of harvest >90%, with healthy cells obtained after harvest

Comparison of 2D vs 3D Cultures for CL-MSCs @ P6

	2D Culture Flask	3D BioNOC™ II
Cell morphology	Polyhedral	Spindle shaped, fibroblastic like
Cell density	2.5 million / T75 flask	2e8 cells / CelCradle™
Media usage	15 ml	500 ml
Cell number: media usage	166,000 cells : 1 ml	400,000 cells : 1ml
To obtain 2e8 cells	80 T75 flasks with 1200 ml media	1 CelCradle™ with 500 ml media
% cells retaining stemness	79%	98%

MSCs are able to achieve ~10 times fold expansion on CelCradle™

Currently seeding between 2-3e7 cells per CelCradle™ to obtain 2e8 cells
Projected to obtain 4e9 cells in 2L TideCell® and 9e11 cells in 300L TideCell®

*Final density will vary based on age, source of stem cells and media type used

ESCO ASTER CDMO SERVICES & TIDE MOTION BIOREACTORS

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