MSC-EVs Manufactured in Scalable 3D Bioreactor Systems

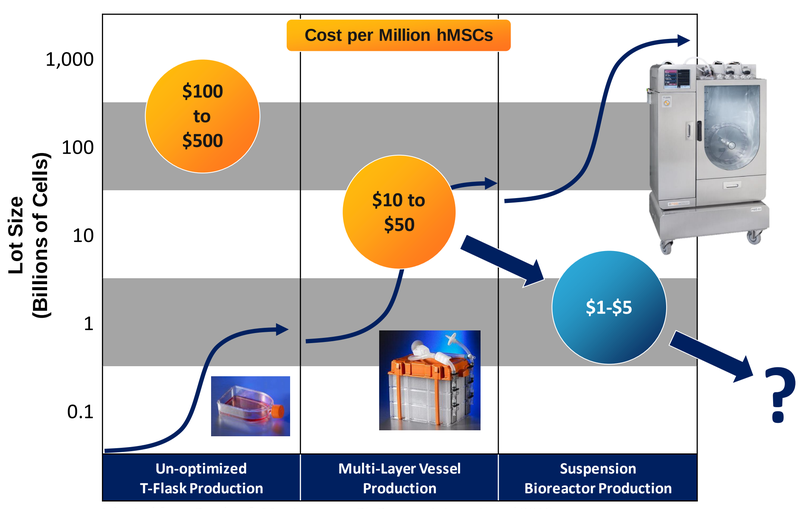
Katrina Adlerz, Michelle Trempel, Jamie Brennan, Jon Rowley, Taby Ahsan

RoosterBio®
Radically Simplifying Use of MSCs

ABSTRACT

- There have been >900 clinical trials using mesenchymal stem/stromal cells (MSCs) for therapeutic applications.
- Due to their similar therapeutic effects to MSCs and potential as a key bioactive agent in regenerative medicine applications, MSC-derived extracellular vesicles (MSC-EVs) are being increasingly investigated as a clinical therapy for a broad range of indications.
- It was recently found that the number of exosomes released from 2M MSCs in 48hrs is equivalent to a single dose for a rodent. Hence, most clinical indications would require an MSC production lot size that is not readily achievable in 2D culture.
- Therefore, larger scalable bioreactor systems will be crucial to generate enough EVs to meet the clinical dose requirement.
- The objective of this study is to:
  - develop a scalable process for xeno-free (XF) MSC-EV manufacturing
  - compare MSC-EV characteristics from 2D culture and various bioreactor scales.
- We demonstrated that this manufacturing process was directly scalable from the small (0.1L) to development (3L) and pilot (15L) bioreactors, maintaining similar cell density and EV productivity at the different BR scales.

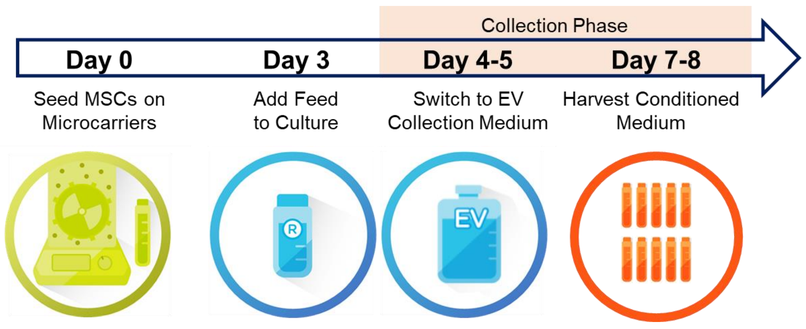
MSC MANUFACTURING PLATFORM EVOLUTION



Adapted from Rowley & Montgomery, *BioProcess International* 2018.

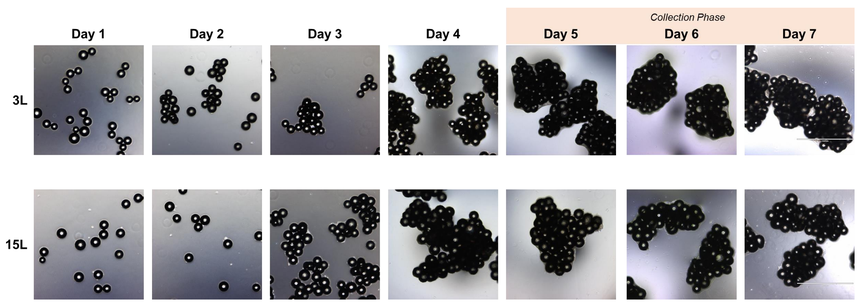
- MSC manufacturing platforms have evolved as the demand for cells increases.
- Suspension bioreactor production of MSCs is a scalable manufacturing platform that can provide the necessary lot sizes of billion to trillions of cells while reducing the time, labor, and cost of goods for regenerative medicine applications.

ESTABLISHED MSC BIOREACTOR EXPANSION PROCESS



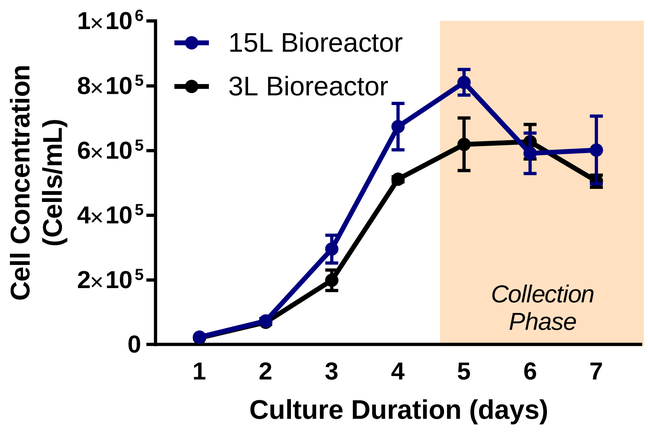
- **Protocol for productions of MSC-EVs**: MSCs are seeded into the bioreactor on Day 0 and cultured in expansion medium (RoosterNourish™-MSC-XF), a bioreactor feed (RoosterReplenish™-MSC-XF) is added on Day 3, and on Day 4-5 of culture (donor dependent) the medium is replaced with a low-particle EV Collection medium (RoosterCollect™-EV). After three additional days of culture, the conditioned medium is harvested.
- Overall, this scalable process collects EVs from MSCs while minimizing the potential of contamination from ancillary materials.

MSCS IN SCALABLE BIOREACTOR CULTURE SYSTEMS



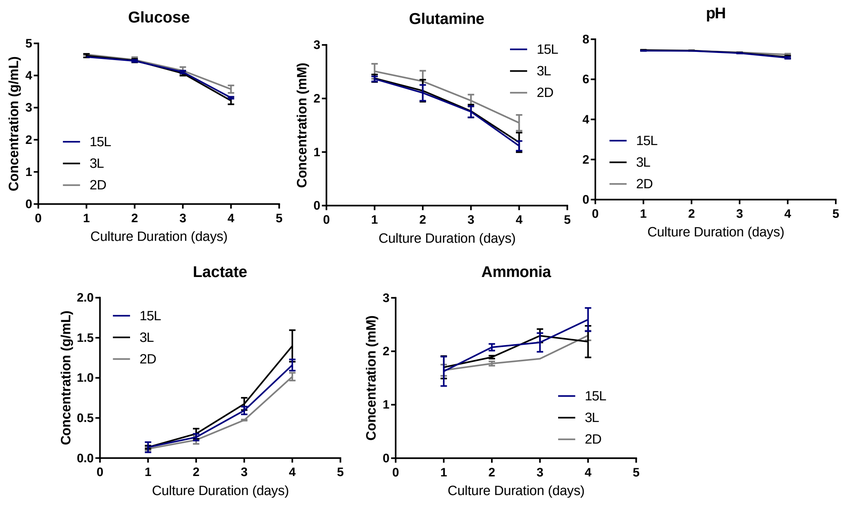
- Bone marrow-derived MSCs were grown in xeno-free, scalable bioreactor culture systems and EVs were collected from the conditioned medium.
- MSC cultures were sampled and monitored for cell growth on microcarriers during cell expansion (Days 1-4) and EV Collection (Days 5-7).
- MSC proliferation was observed by the formation of cell-based aggregates during expansion. The distribution of cells on microcarriers throughout the bioreactor culture was comparable across scales, demonstrating a scalable seeding strategy.

COMPARABLE MSC GROWTH IS ACHIEVABLE ACROSS BIOERACTOR SCALES



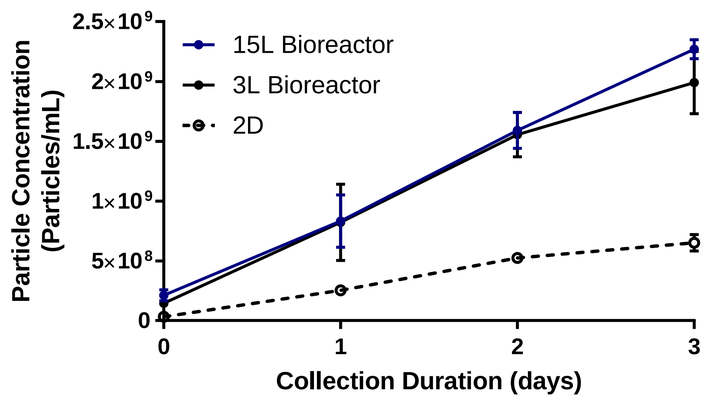
- The bioreactor culture process has been successfully scaled to the 3L development and 15L pilot scale (n=2 shown).
- Results show similar growth profiles among the scales with increasing cell concentrations for the first four days when the cells are in the expansion phase.
- At Day 4, the medium is switched to an EV collection medium and cell number remained stable for 3 days.

COMPARABLE NUTRTIENT / WATE / PH PROFILES



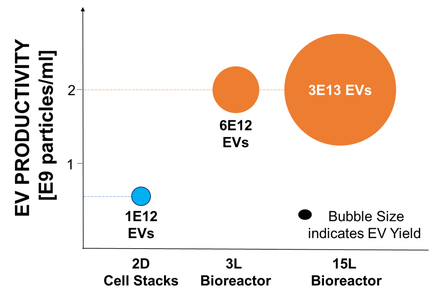
- The concentrations of glucose and glutamine were maintained at appropriate levels throughout culture to support cell expansion in both 2D, development bioreactor (3L) and pilot bioreactor (15L) systems.
- Build up of lactate and ammonia was similar between bioreactor and 2D culture using a xeno-free fed-batch bioreactor process.

PARTICLE YIELD IS INCREASED IN BIOREACTOR CULTURE



- Particle concentration in the conditioned medium increased over three days in EV collection medium.
- The bioreactor systems exhibited a greater particle concentration compared to the 2D flask system.

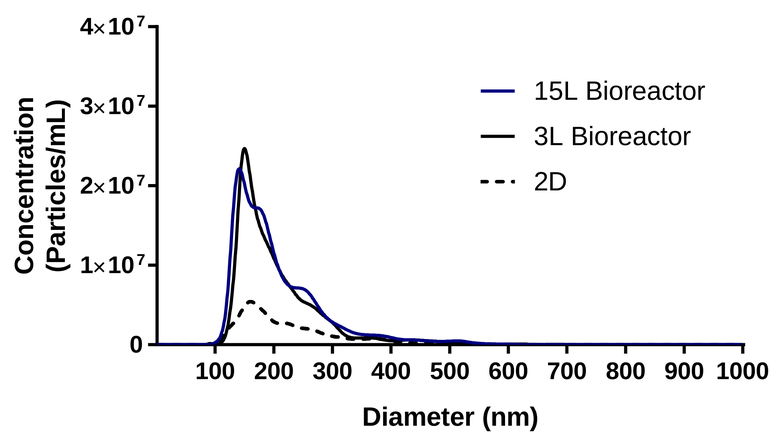
CELL AND PARTICLE YIELD ACROSS SCALES



Culture System	Cell Density at Switch	Theoretical Total Cells	Particle Yield
2D CS10 (n=2)	93,300 cells/cm^2	0.5 Billion	9.77 E11
3L (n = 2)	511,000 cells/ml	1.5 Billion	5.97 E12
15L (n = 2)	674,000 cells/ml	10 Billion	3.41 E13

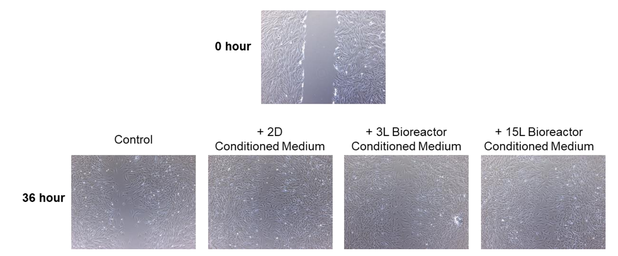
- As would be expected, higher volume systems lead to greater cell yields, and therefore particle yields.
- Bioreactor systems, however, also support a greater EV productivity level, as measured in particles/cell.
- As the bioreactor systems were scaled up, the higher levels of EV productivity remained and yield increased proportionally.

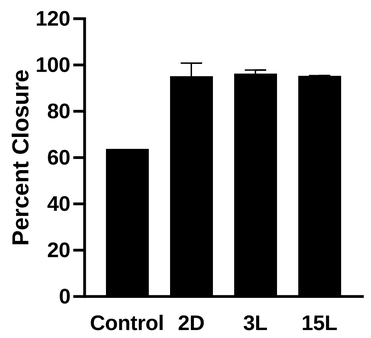
COLLECTED PARTICLES ARE SIMILAR ACROSS SCALES



- For both bioreactor systems, as well as the 2D culture system, the size distributions as determined by Nanoparticle Tracking Analysis (Malvern NanoSight) showed that the particles collected from the conditioned medium were consistent with extracellular vesicles.

CONDITIONED MEDIIUM FROM 2D AND 3D CULTURE ALL EXHIBIT BIOACTIIVTY IN A WOUND HEALING ASSAY



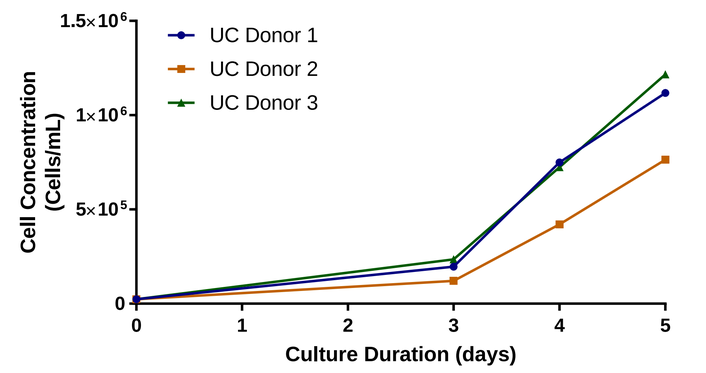


- Conditioned medium from the 2D culture, 3L and 15L Bioreactors all showed bioactivity in a wound healing assay and increased wound closure compared to the control of EV collection medium alone.

SUMMARY

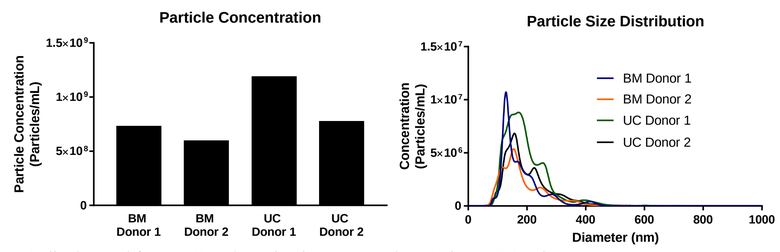
- We developed a scalable fed-batch process for large-scale expansion of MSCs for EV production in suspension bioreactors that yields consistent EVs.
- Rapid clinical translation is possible with this system using RoosterBio CliniControl™ products.
- Further work in maximizing EV yields in this scalable systems will allow for generating lot sizes for clinically relevant doses of MSC-EVs.

BIOREACTOR CULTURE WITH UC-MSCS



- While the bioreactor scale-up data shown used bone marrow-derived MSCs (BM), MSCs can be isolated from other tissue sources as well.
- Here, cells derived from umbilical cord (UC) tissue were expanded in 0.1L bioreactors. UC-MSCs reached higher cell concentrations and expanded faster than bone marrow-derived MSCs.

PARTICLE GENERATION WITH UC-MSCS



- Cells derived from UC and BM both generated particles in 2D culture.
- The particle concentration in the conditioned medium was higher in the two UC cultures than the BM cultures. Higher cell densities of the UC-MSCs may explain this increase. Indeed calculated particles/cell was similar or slightly higher for BM-MSCs than UC-MSCs.
- The particle size distribution shows particle diameter was similar between BM and UC MSCs and consistent with EVs.
- These results demonstrate that MSCs from multiple tissue sources could be used for MSC-EV production. Future experiments will scale UC-MSC culture and EV production in bioreactor culture.