Xeno-Free Manufacturing of MSC-EVs in Scalable Bioreactor Culture

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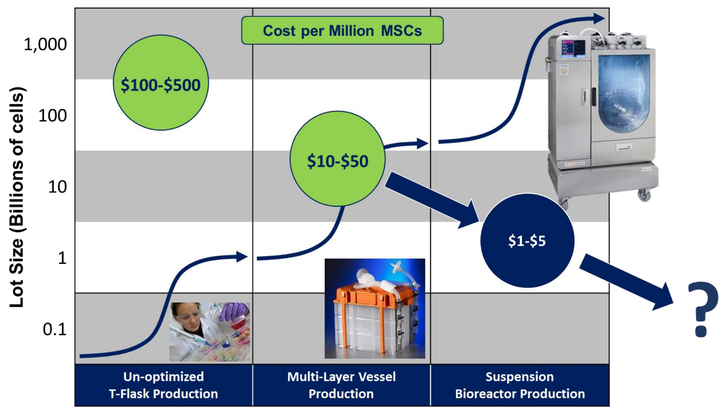
ABSTRACT

- 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 indications would require a MSC production lot size that is hardly achievable in 2D culture.
- Therefore, larger scalable bioreactor systems will be crucial to generate enough EVs to meet the clinical dose requirement.
- This study developed a process for xeno-free (XF) scalable MSC-EV manufacturing and compared MSC-EV characteristics from 2D culture and various bioreactor scales.
- We demonstrated that this process was directly scalable from the small (0.1L) to development (3L) and pilot scale (15L) bioreactors, maintaining similar cell density. To remove the residual particles from the expansion media, an additional wash step before the switch to the collection media was required in bioreactors.
- With a similar collection process and comparable cell density, we also observed consistent EV production at these different scales of manufacturing systems.

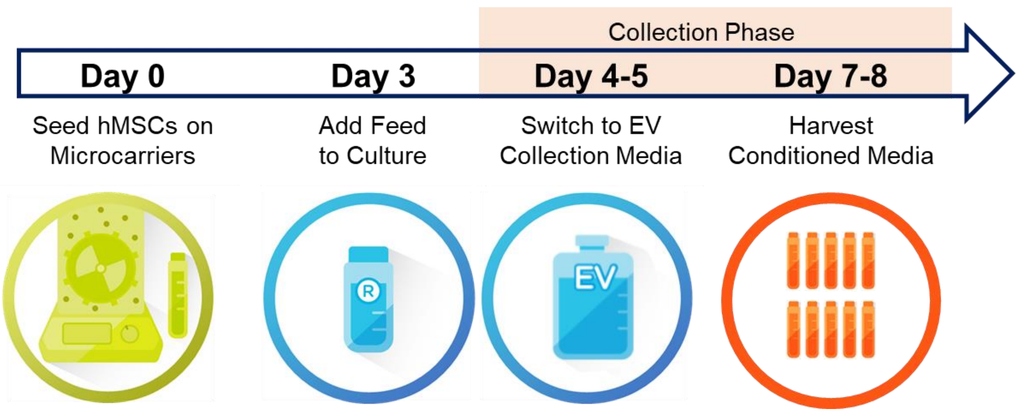
hMSC MANUFACTURING PLATFORM EVOLUTION



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

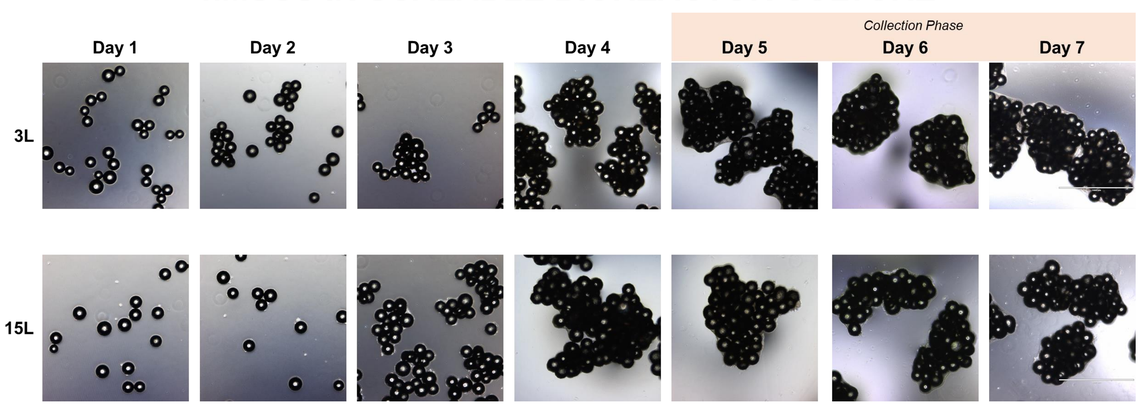
- hMSC manufacturing platforms have evolved as the demand for cells increases.
- Suspension bioreactor production of hMSCs is a scalable manufacturing platform that can provide the necessary lot sizes of billion to trillions of cells while reducing the time, labor, and cost of goods for regenerative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

ESTABLISHED hMSC BIOREACTOR EXPANSION PROCESS



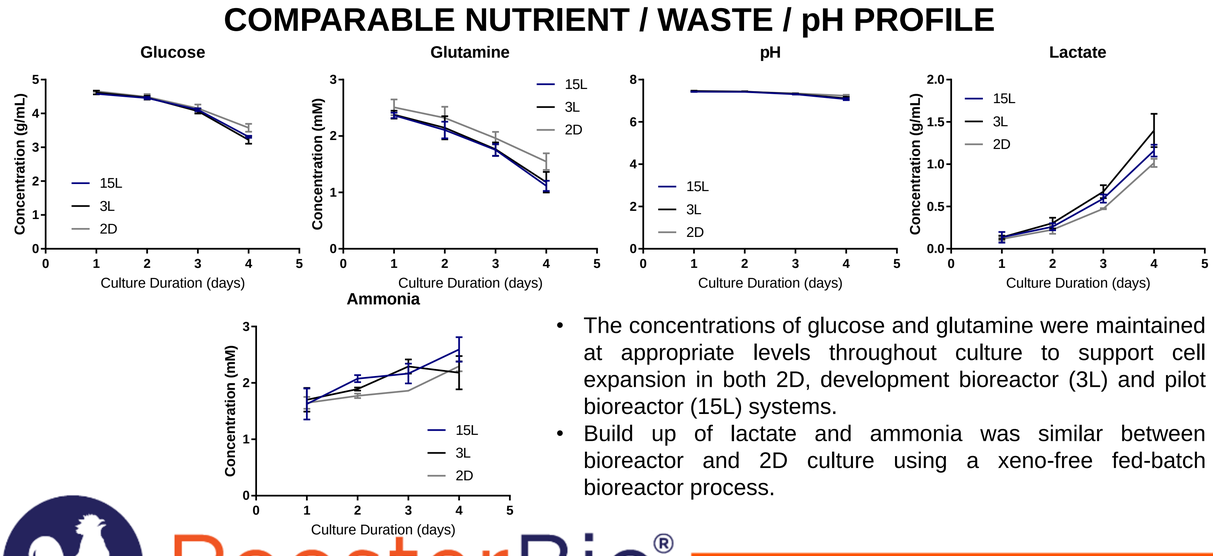
- A protocol was established for the production of hMSC-EVs.
- hMSCs are seeded into the bioreactor on Day 0, a bioreactor feed (RoosterReplenish™-MSC-XF) is added on Day 3, and on Day 4 or 5 of culture the expansion media (RoosterNourish™-MSC-XF) is replaced with a low-particle EV Collection media (RoosterCollect™-EV). After three additional days of culture, the conditioned media is harvested.
- This process provides confidence that the collected EVs are derived from the cultured cells and not ancillary materials.

hMSCS IN SCALABLE BIOREACTOR CULTURE



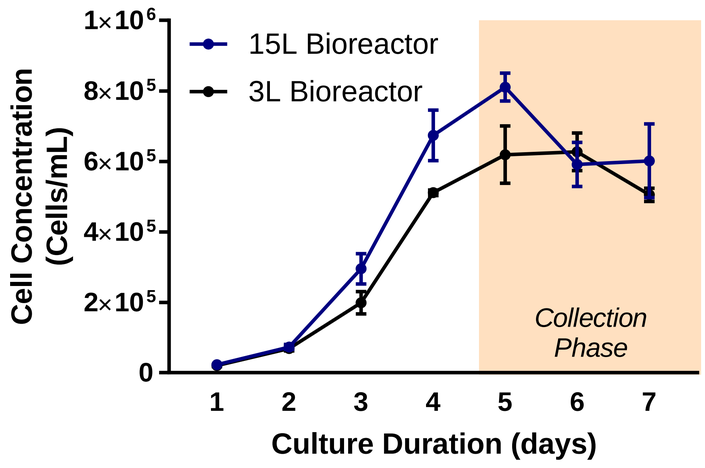
- hMSCs were grown in xeno-free, scalable bioreactor culture systems and extracellular vesicles were collected from the conditioned media.
- hMSC cultures were sampled and monitored for cell growth on microcarriers during cell expansion (Days 1-4) and EV Collection (Days 5-7).
- hMSC proliferation was 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 NUTRIENT / WASTE / pH PROFILE



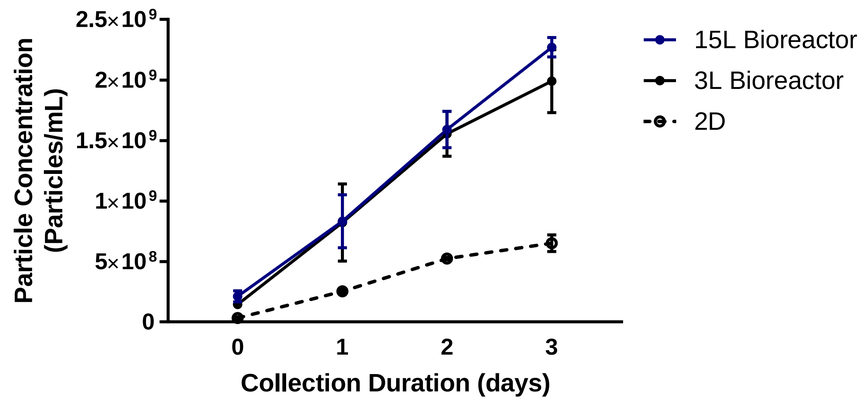
- The concentrations of glucose and glutamine were maintained at appropriate levels throughout culture to support cell expansion in both 2D, development bioreactor (3L) and pilot bioreactor (15L) systems.
- Build up of lactate and ammonia was similar between bioreactor and 2D culture using a xeno-free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

COMPARABLE hMSC GROWTH PROFILE ACROSS BIOREACTOR SCALES



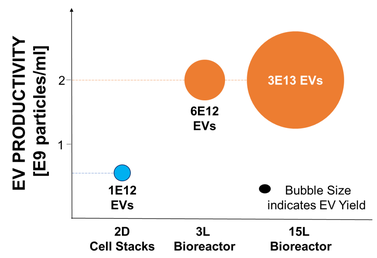
- The bioreactor culture process has been successfully scaled to the 3L development scale (n=2) and 15L pilot scale (n=2).
- Results show similar growth profiles among the scales with increasing cell concentrations for the first four days when the cells are in the expansion phase.
- At Day 4, the media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

PARTICLE YIELD IS INCREASED IN BIOREACTOR CULTURE



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

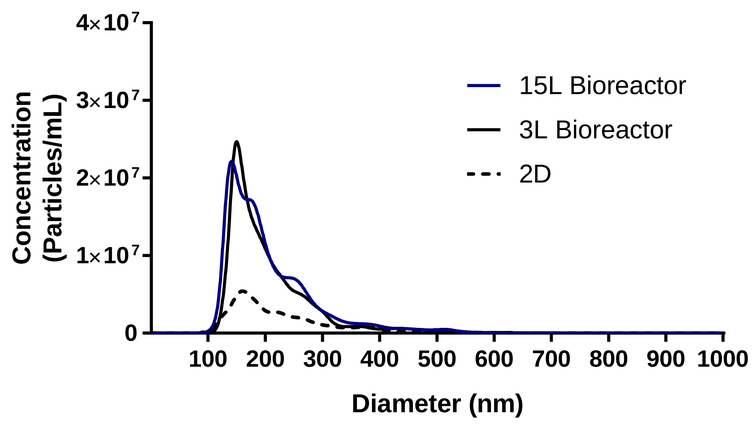
CELL AND PARTICLE YIELD ACROSS SCALES



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

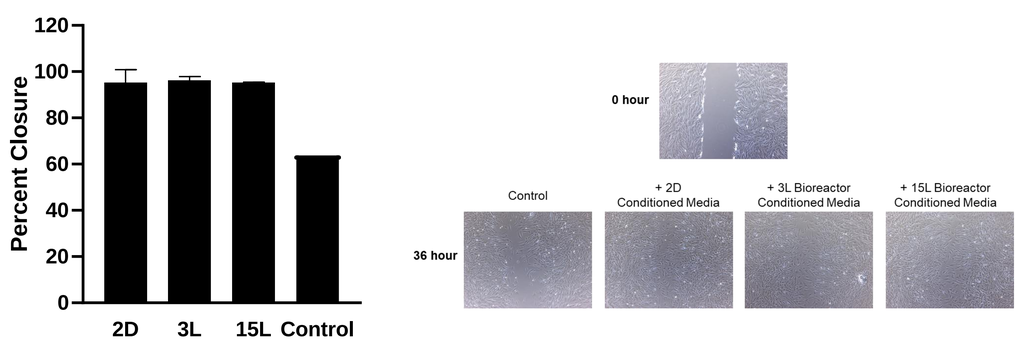
- Bioreactor culture systems generate higher cell yields and higher particle yields compared to 2D culture systems.
- Bioreactor systems support a greater EV productivity level, as measured in particles/cell.

COLLECTED PARTICLES ARE SIMILAR ACROSS SCALES



- For both bioreactor systems, as well as the 2D culture system, the size distributions show that the particles collected from the conditioned media are consistent with extracellular vesicles.

CONDITIONED MEDIA FROM 2D AND 3D CULTURE ALL EXHIBIT BIOACTIVITY



- Conditioned media 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 media alone.

SUMMARY

- Optimizing EV yield is become increasingly important as EVs are being developed as clinical therapies.
- We have developed a fed-batch process for large-scale expansion of hMSCs and a protocol for EV production in suspension bioreactors that is scalable to 15 L and yields consistent EVs.
- Rapid clinical translation is possible with this system using RoosterBio CliniControl™ products.
- We intend to continue to scale this process up to the production scale (80L) bioreactors, which is critical to generating lot sizes for clinically relevant doses of MSC-EVs.