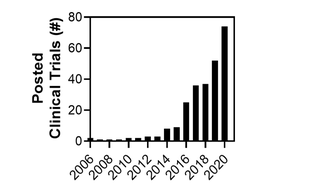
Developing a microcarrier stirred tank process for large-scale hMSC-EV production

Stephen Lenzini, James Brennan, Elie Zakhem & Jon Rowley

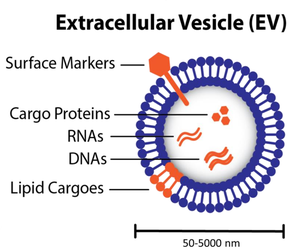
RoosterBio®
Radically Simplifying Use of MSCs

Background & Objective

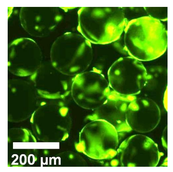
- Extracellular vesicles (EVs) are nano-sized particles composed of a lipid bilayer with promising therapeutic potential.
- EVs are released from many different types of cells, and they contain proteins, nucleic acids (RNA and DNA), and lipid cargoes.
- EVs released from Mesenchymal Stromal/Stem Cells (hMSC-EVs) are rapidly gaining momentum in clinical trials.
- One of the challenges in EV production is scaling up the manufacturing processes using the optimal cell source and collection media.
- RoosterBio offers reproducible and scalable processes to manufacture high-quality, xeno-free (XF) hMSCs and media for EV production.
- EV productivity is determined by the final cell yield and the number of EVs generated per cell. Therefore, increasing final cell yield as a first step will increase EV productivity.
- **Objective:** The goal of this study was to develop an optimized scalable process for MSC expansion and EV production in a stirred tank bioreactor system.

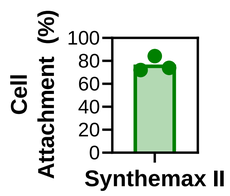


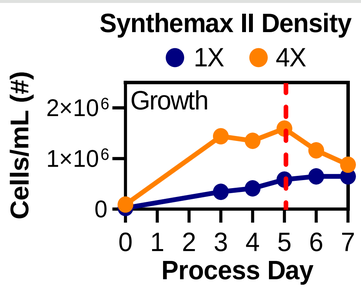
Clinical trials investigating EVs as therapeutics or readout material. From clinicaltrials.gov query: "extracellular vesicles" OR "exosomes". Search on 17Jun2021.



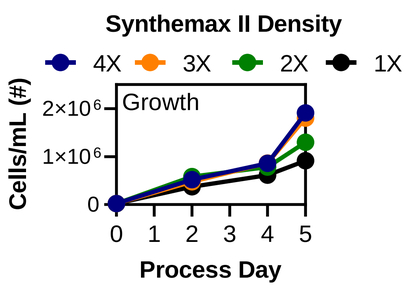
Maximizing cell density in Bioreactors

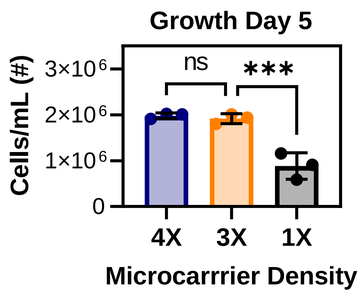




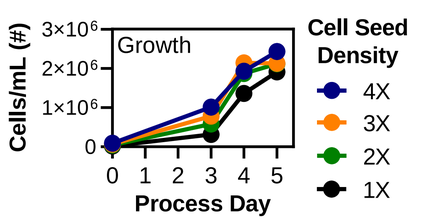


Synthemax® II (Corning®) showed high MSC attachment potential (~80%) and was used for the rest of the study. MSC growth reaches its peak around day 5 of culture and then plateaus after that, therefore 5 days of growth was selected as the optimal parameter for growth phase length.



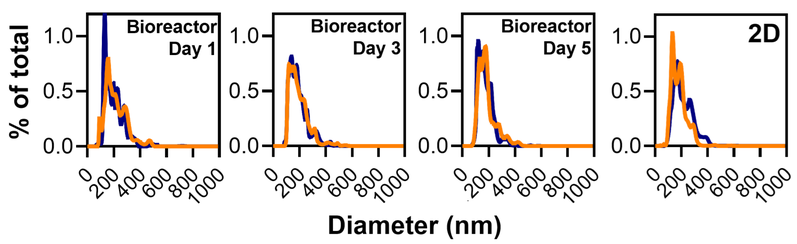


As the density of microcarriers increased (from 1X to 4X), final cell yield increased by day 5 of culture (*** $p<0.05$, one-way ANOVA with multiple comparisons). 4X microcarriers did not yield higher cell number compared to 3X (ns = not significant).

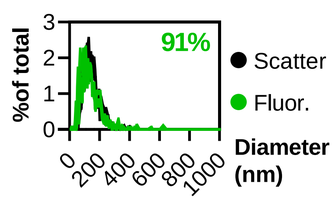


Initial cell seeding density at 1X yielded similar final cell number regardless of the microcarrier concentration used, therefore a combination of 1X cell seeding density and 3X microcarrier density was selected as the optimal condition.

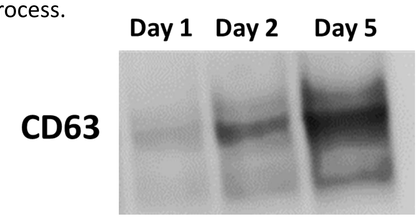
Maintenance of EV Critical Quality Attributes



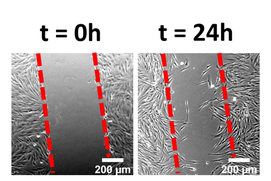
hMSC-EVs that were collected from RoosterCollect-EV™ showed consistent size distribution over the period of collection with diameter ~50-500nm and median ~180nm. The size distribution was consistent with a 2D control process.

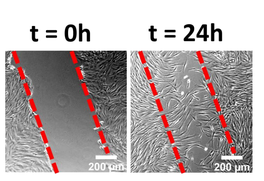


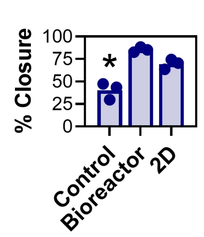
hMSC-EVs harvested from this process showed ~91% positive with a membrane-bound fluorescent dye, indicating intact vesicles with consistent size distribution.



Western blot performed on hMSC-EVs collected from the bioreactor showed positive stain for tetraspanin marker CD63, confirming the identity of the collected EVs.

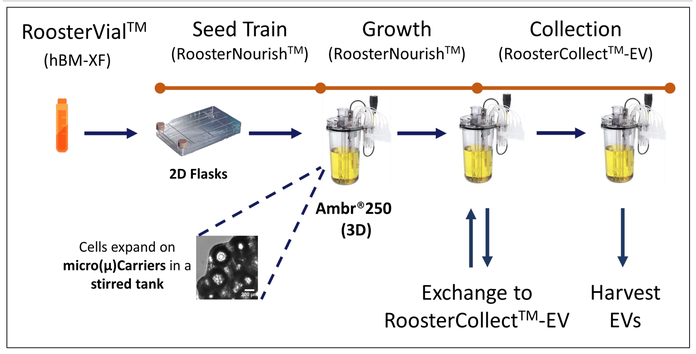






In vitro wound healing assay was performed to assess the potency of the harvested EVs. EVs collected from the bioreactor enhanced migration of fibroblasts and achieved ~80% closure of the created wound similar to 2D. Control media (without EVs) achieved around 40% closure. *$p < 0.05$ vs. other groups via one-way ANOVA.

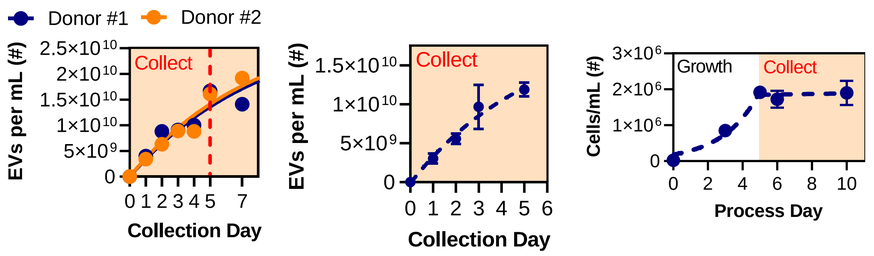
Determining Critical Process Parameters



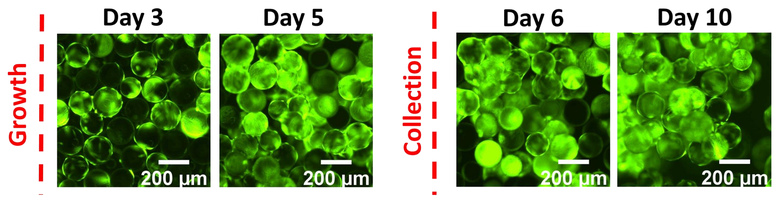
Process Flow Diagram showing the adapted process for EV manufacturing in stirred tank bioreactor

- This study was carried out using xeno-free human bone marrow MSCs (RoosterVial™-hBM, RoosterBio) expanded in RoosterNourish™ (RoosterBio) in Ambr®250 (Sartorius). EVs were collected in low-particle media, RoosterCollect™-EV (RoosterBio).
- **Critical process parameters (CPPs)** were evaluated and optimized to increase final cell yield and EV productivity:
  - ❑ Microcarrier type
  - ❑ Cell growth phase length (pre-collection)
  - ❑ Microcarrier density
  - ❑ Initial cell seeding density
  - ❑ EV Collection time
- **Critical quality attributes (CQAs)** were evaluated for count and size (Nanosight NS300), identity (tetraspanins by western blot) and potency (in vitro wound healing assay).

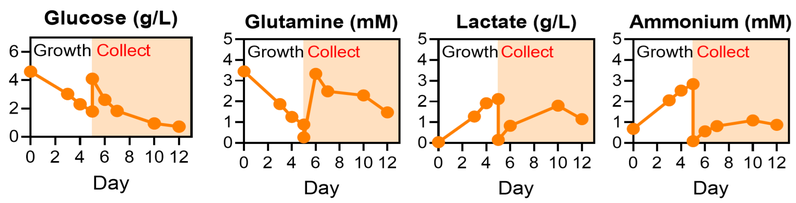
hMSC-EV Collection Phase Evaluation



After 5 days of cell growth, RoosterNourish™ was replaced with the low particle media RoosterCollect™-EV and EV yield was followed over 7 days. EV production reaches peak near 5-7 days (1 to $2x10^{10}$ EV/mL). Thus, 5-day collection is sufficient and can be extended up to 7 days (N = 3 experiments and 2 donors, data fit with one-phase association). Cell number was maintained during EV collection phase.

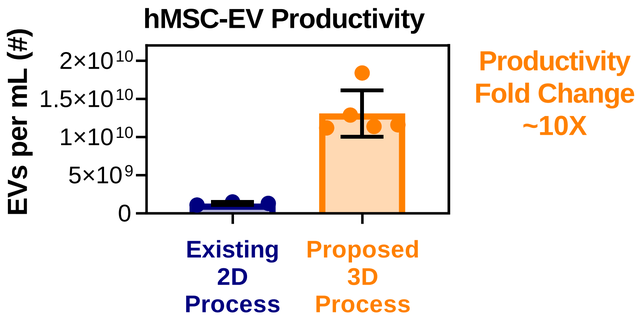


Microcarrier Synthemax II supported MSC attachment and growth as shown by Calcein AM live stain during cell growth and EV collection phases.

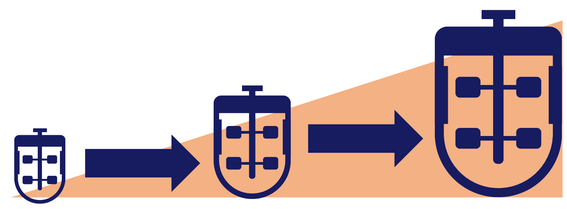


Metabolites follow expected behavior over the growth and collection phases.

Conclusion



- In this study, we successfully developed and optimized a process to maximize hMSC (RoosterVial™-hBM) yield using RoosterNourish™ and microcarriers in a stirred tank bioreactor system (Ambr®250, Sartorius).
- We also successfully optimized the process for maximal EV productivity in RoosterCollect™-EV to reach $1-2x10^{10}$ EV/mL.
- Critical process parameters included microcarrier type, growth phase time, microcarrier density, cell seed density, and collection phase time.
- Process reproducibility was verified across three independent experiments and two tissue donors.
- EVs harvested from the bioreactor maintained the critical quality attributes compared to the 2D process, including size distribution and maintenance of biofunctionality (in vitro wound closure).
- Further work will be performed to understand CQA expression as a function of collection day. These results will further inform the collection phase CPPs.
- Future studies will focus on scaling up this process to larger volumes.



5295 Westview Drive, Suite 275, Frederick, MD 21703 USA
www.roosterbio.com