

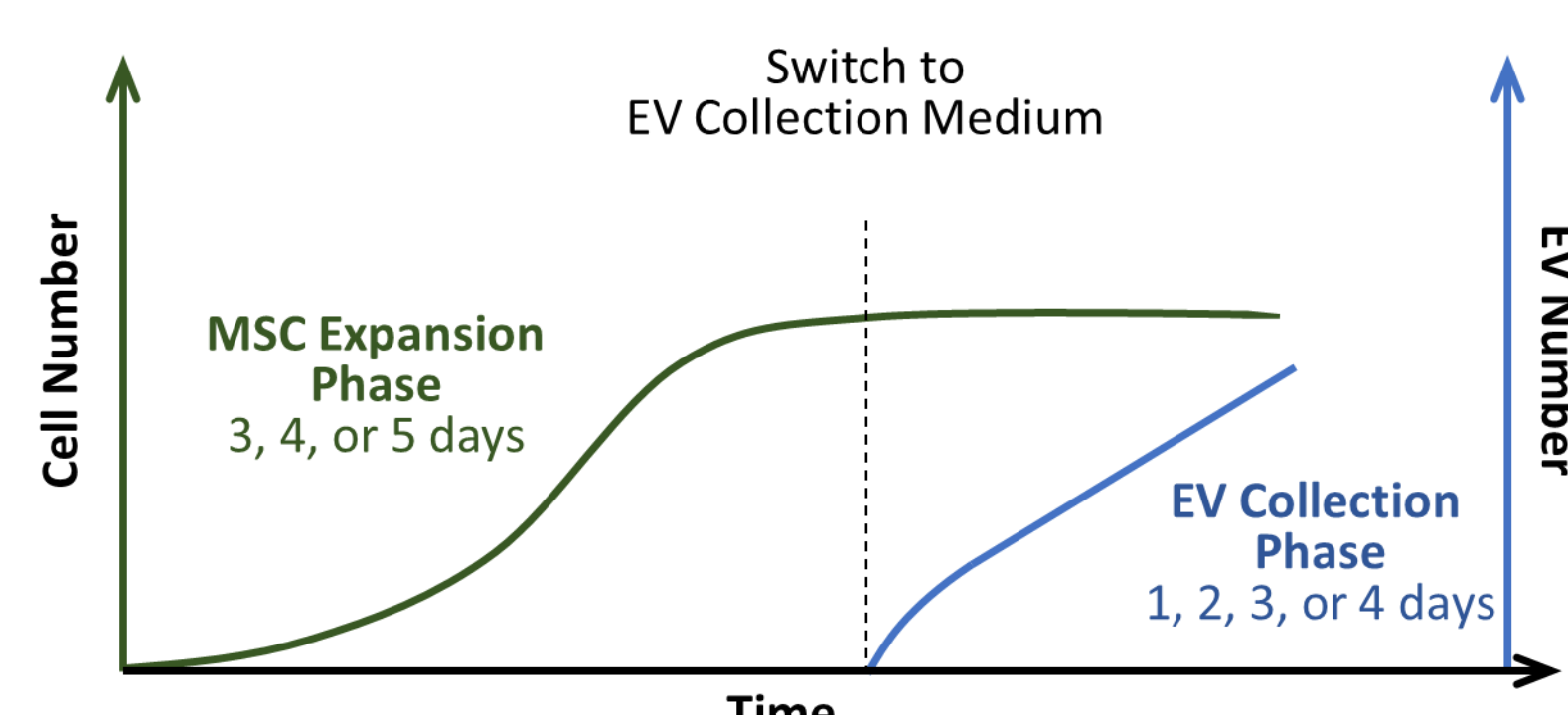
Increasing Yield Of MSC-EVs in Scalable Xeno-Free Manufacturing

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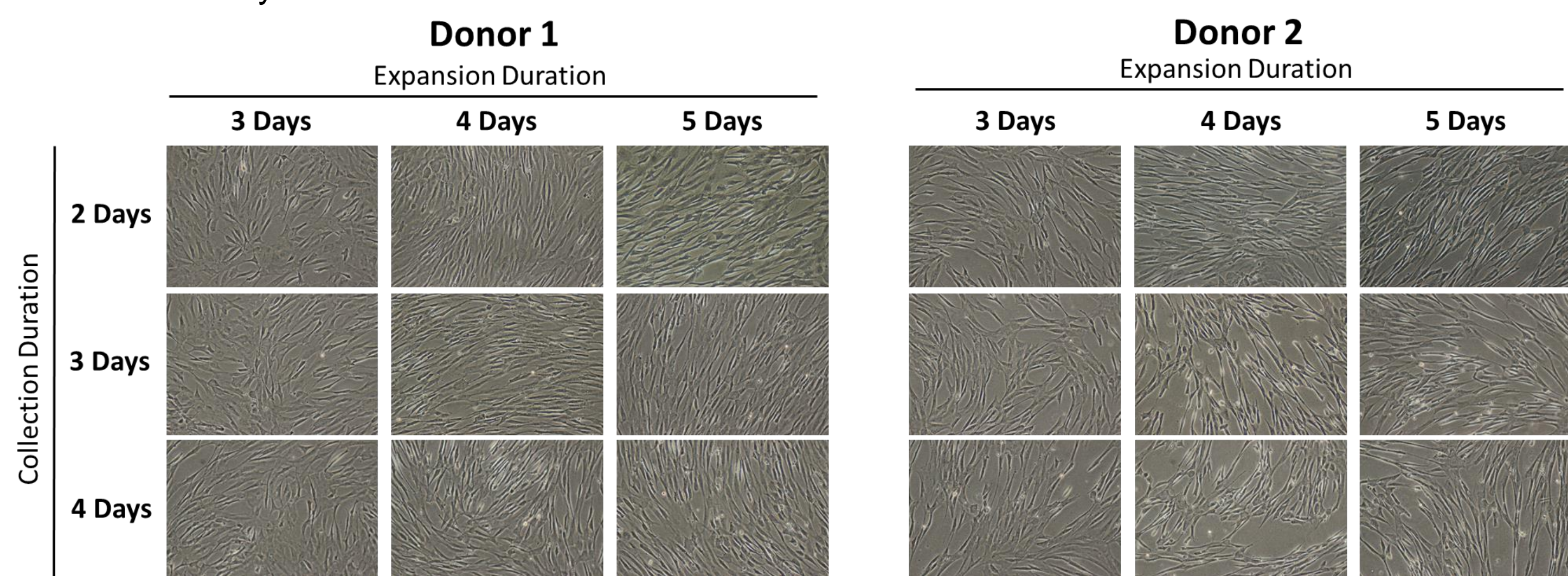
INTRODUCTION

- Extracellular vesicles (EVs) are released from many different cell types. They can contain both proteins and nucleic acids inside a lipid membrane that serves to protect the cargo.
- EVs are increasingly being investigated as both a cell-free therapy and a drug delivery vehicle.
- In particular, mesenchymal stem/stromal cells (MSCs) have been used in over 900 clinical trials, and offer a well-studied cell source to generate EVs.
- A critical barrier in the development of MSC-EVs as a commercial therapy, however, is generating the large amount of EVs that will be required per dose.
- In this study, two strategies were investigated to maximize EV yield:
 - Optimize MSC (human bone-marrow derived) culture duration and EV collection duration
 - Increase EV productivity by adding EV Boost™ during the EV collection period.

PARAMETER EVALUATION FOR AN EV COLLECTION PROCESS FROM MSCs

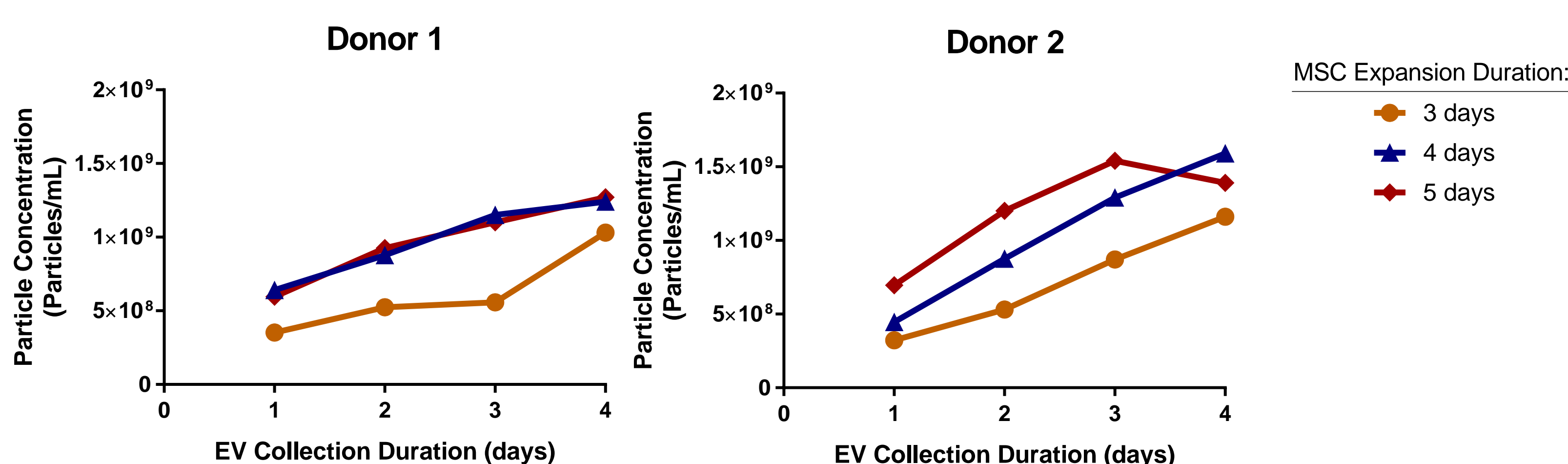


- Human bone-marrow derived MSCs were passaged once from a working cell bank, then expanded for an additional passage for 3, 4, or 5 days.
- After the cell expansion phase, the expansion medium (RoosterNourish™-MSC-XF) was replaced with a low-particle EV collection medium (RoosterCollect™-EV) and the conditioned medium was collected after 1, 2, 3, or 4 days.
- Overall, this process paradigm collects EVs from MSCs while minimizing the potential of contamination from ancillary materials.

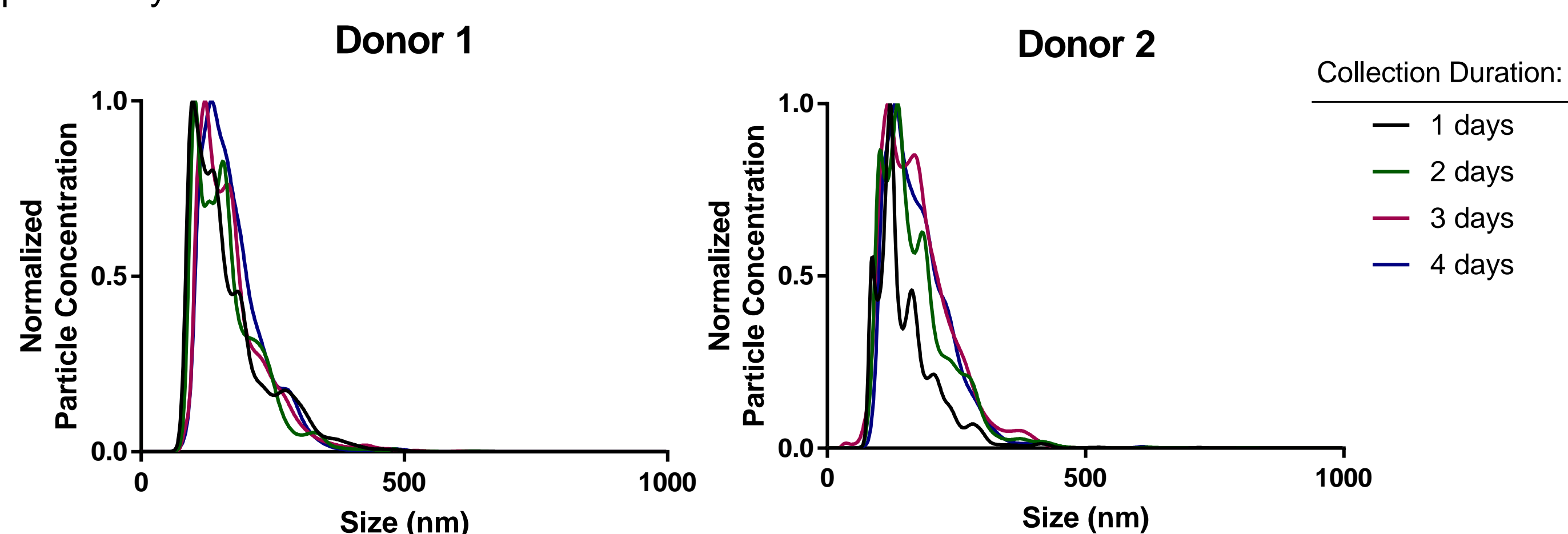


- Utilizing the process described above, cells exhibited a typical MSC morphology for up to 4 days in the collection medium RoosterCollect-EV.

PARTICLE CONCENTRATION INCREASES WITH INCREASING CELL NUMBER OR COLLECTION DURATION

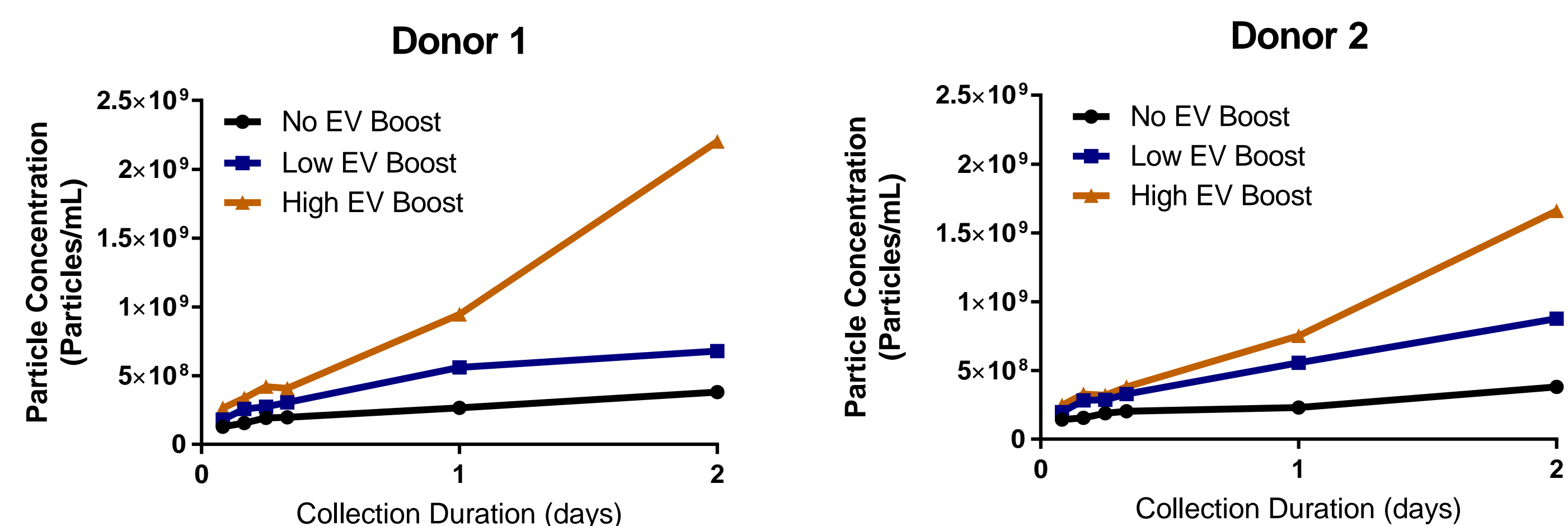


- Particle concentration in the conditioned medium was measured by Nanoparticle Tracking Analysis using a Malvern NanoSight.
- Particle concentration was greater when the attached cell density was higher, as controlled by increased MSC expansion duration prior to EV collection.
- Cumulative particle concentration also continued to increase with extended EV collection durations for up to 4 days.

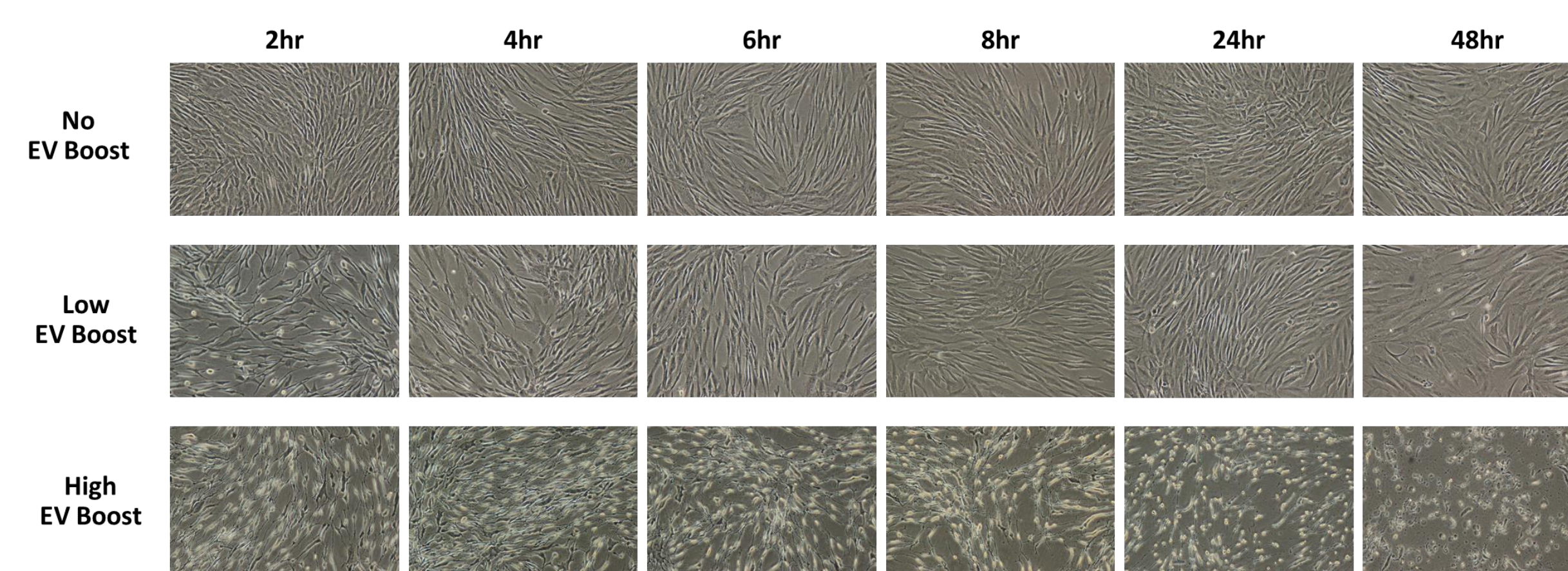


- The size distribution of the collected particles for all test conditions in this study were consistent with the expected diameter of extracellular vesicles.
- Based on these results, a process was adopted to generate a high EV yield from the highest cell number with a shorter EV collection duration:
 - cells are passaged once from a working cell bank vial and then expanded to confluency
 - culture is switched to low-particle medium (RoosterCollect™-EV)
 - EVs are collected in the conditioned medium for 2 days.

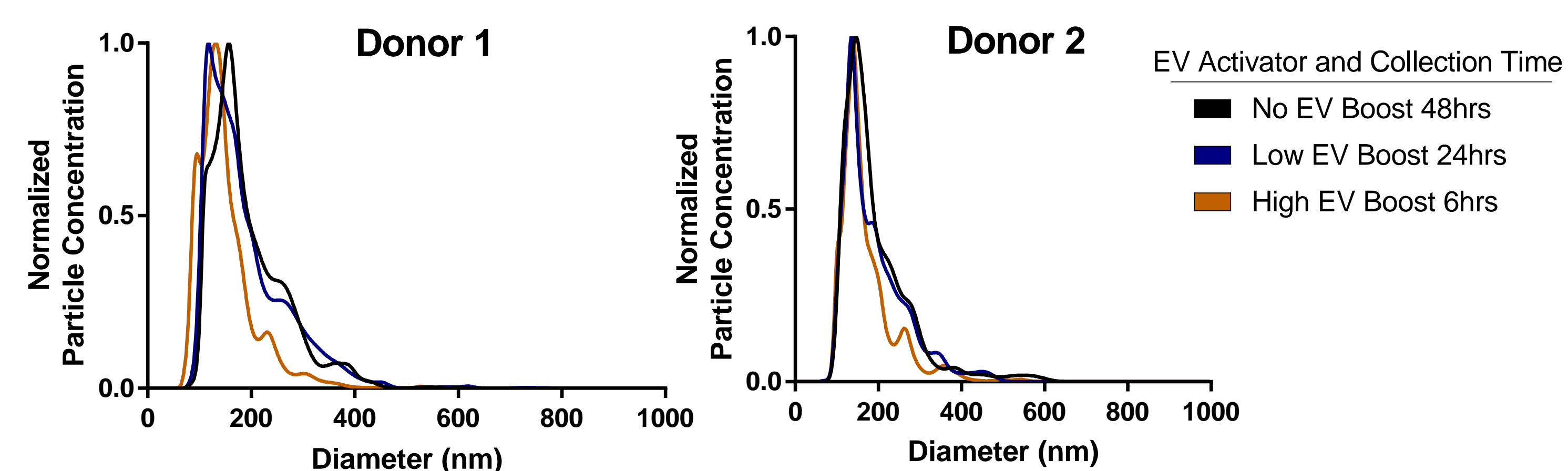
EV BOOST™ ACTS TO INCREASE YIELD AND SHORTEN COLLECTION TIME



- EV Boost is a medium supplement that is added to RoosterCollect-EV and acts as an EV activator to collect more particles from cell culture.
- Culture of MSCs in EV collection medium with either a low or high concentration of EV Boost (Low EV Boost and High EV Boost, respectively) resulted in higher particle concentrations compared to conditions in RoosterCollect-EV alone (No EV Boost). The Low and High EV Boost conditions also resulted in more EVs sooner, allowing for shorter collection durations with similar output compared to No EV Boost.

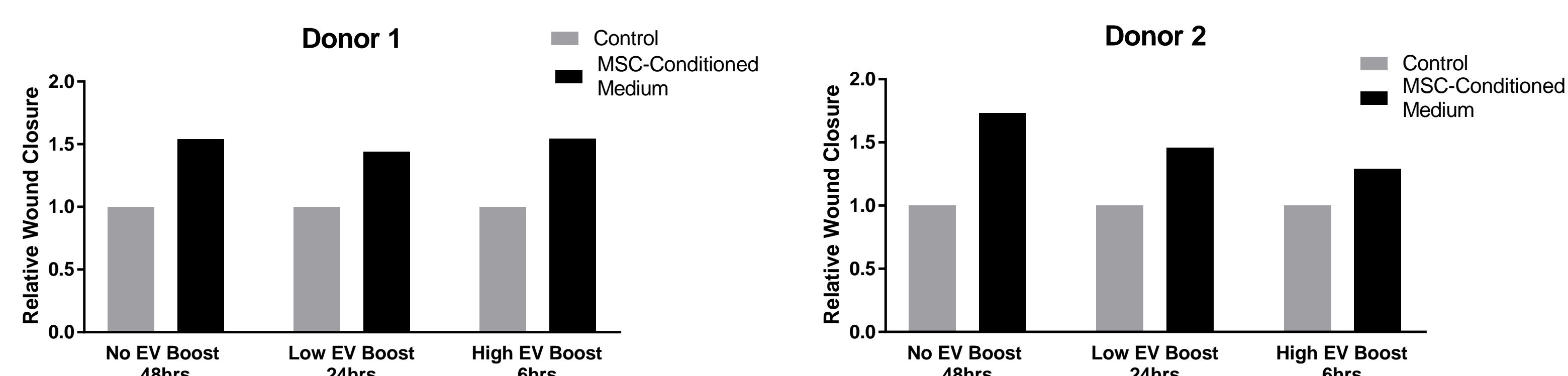


- MSCs maintained cell morphology for at least 48hrs and 24hrs in No EV Boost and Low EV Boost, respectively. High EV Boost, not meant for extended cell cultures, influenced cell morphology and cell attachment at the longer culture durations tested.
- Based on these results, High EV Boost was used for 6hrs and Low EV Boost was used for 24hrs to generate EVs for further characterization.



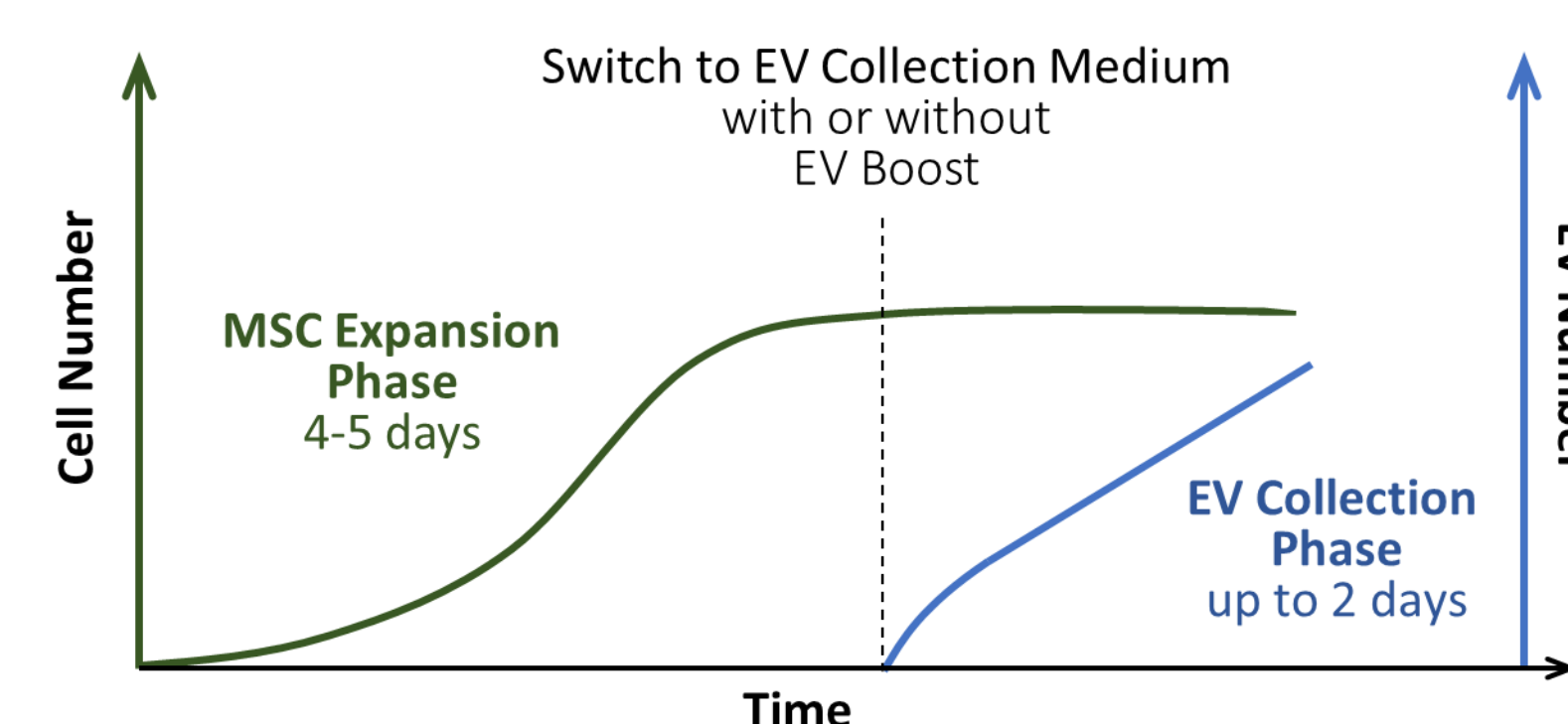
- Using EV Boost as an EV activator added to medium generates particles in the size distribution consistent with extracellular vesicles for all conditions, with select time points shown.

CONDITIONED MEDIUM USING ROOSTERCOLLECT-EV BOTH WITH AND WITHOUT EV BOOST ALL EXHIBIT BIOACTIVITY



- Conditioned medium from cultures with RoosterCollect-EV, with or without supplementation with EV Boost, all exhibited bioactivity and showed increased wound closure compared to the controls in an *in vitro* wound healing assay.

PROCESS FOR MSC-EV PRODUCTION



SUMMARY

- We have developed a process using a xeno-free manufacturing platform that could be scaled to generate the dose sizes of MSCs and EVs necessary for clinical trials.
- EV Boost, an activator of EV generation, increased yield using shorter collection durations.
- To further increase yield, additional EV activators and culture systems (e.g. bioreactors) will be investigated.
- Finally, rapid clinical translation is possible with a RoosterBio system, using the CliniControl™ family of products.



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