Scalable Xeno-Free Manufacturing of Extracellular Vesicles Derived from Human Mesenchymal/ Stromal Stem Cells

Kelvin S. Ng, Prarthana Ravishankar, Robert D. Kirian, Jon A. Rowley , Lye Theng Lock. RoosterBio, Inc. 5295 Westview Drive, Suite 275, Frederick, MD 21703.

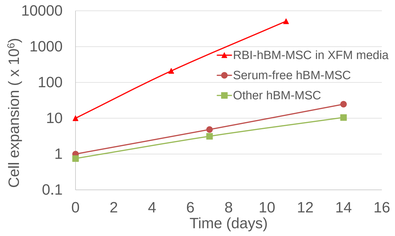
ABSTRACT

Having been investigated in >800 clinicial trials without significant adverse events, human mesenchymal/ stromal stem cells (hMSCs) are a safe and clinically relevant cell source for producing extracellular vesicles (EVs) such as exosomes. Not only can hMSC-EVs deliver exogenous agents including proteins and RNA, hMSC-EVs also inherit the therapeutic potential of hMSCs and have been applied in >20 disease models. However, based on the current state of the art, a single hMSC-EV dose would require an equivalent of >10 hMSC doses to generate, rendering hMSC-EV therapy cost-prohibitive.

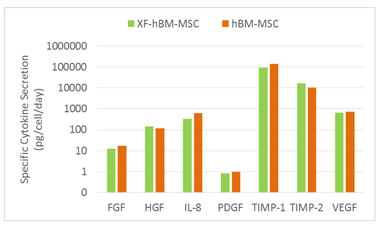
Typical EV production today involves (1) an initial expansion phase where hMSCs are grown in serum-containing medium; (2) buffer exchange where exogeoous EVs in the serum-containing medium are washed off and an EV-free collection medium is added; and (3) an EV collection phase where hMSC-EVs accumulate in the EV-free medium. We hypothesize that the **cost and yield of producing hMSC-EVs can be optimized by improving the expansion phase to achieve more cells and hence more EVs**. To this end, we designed a clinically relevant process utilizing high-volume, xeno-free hMSCs and media to optimize hMSC-EV yield.

RAPID EXPANSION OF HIGH DENSITY, HIGH VOLUME XENO-FREE HBM-MSC

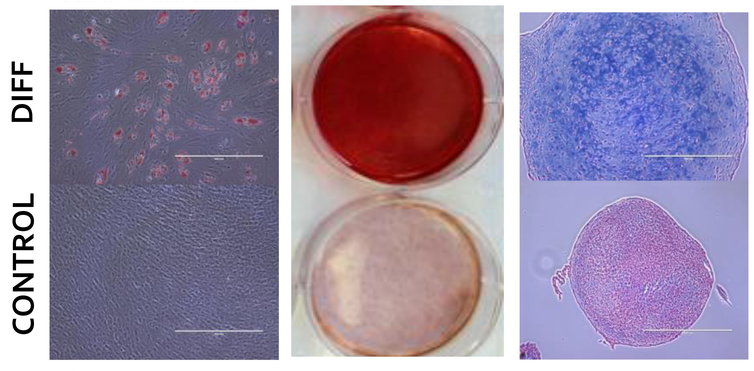
I. Expansion profile of hMSC



II. Cytokine Secretion



III. Tri-lineage Differentiation



IV. Surface Marker Expression

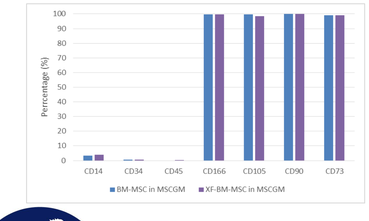


Figure 1: XF hBM-MSC outperform other serum and serum-free hMSC system for their (I) Expansion rate and yield, and maintained their potency and functionality based on (II) Angiogenic cytokine secretion, (III) Tri-lineage differentiation and (IV) Stem cell surface marker expression.

ECONOMIC ADVANTAGE OF ROOSTERBIO hMSC XF BIOPROCESSING SYSTEMS

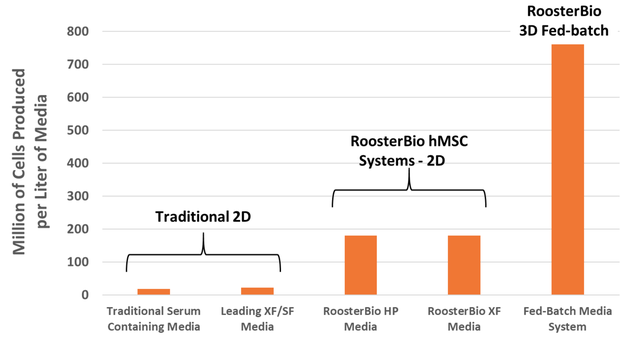


Figure 2: The high efficiency xeno-free media demonstrate that both the 2D and 3D XF systems outperform traditional culture in terms of cells produced per volume of media consumed.

ACCELERATED PROCESS FOR HMSC-EV COLLECTION

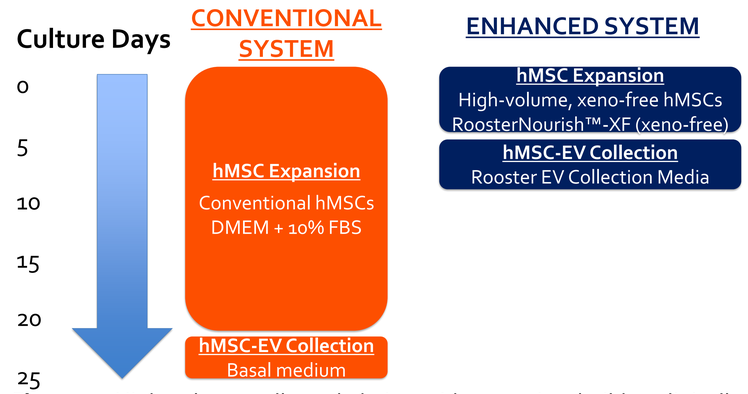


Figure 3: High-volume cells and their rapid expansion build a clinically relevant process for enhanced EV production.

CASE STUDY 1: PAIRED SYSTEM FOR MSC-EV COLLECTION

I. Cell Yield

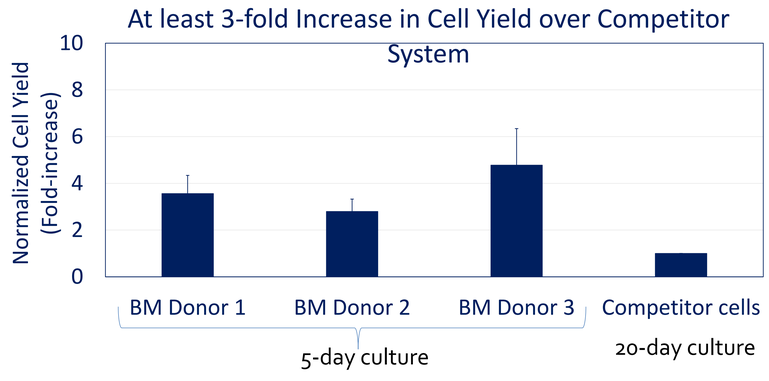


Figure 4: hMSC from three different bone marrow (BM) donors were expanded in hMSC RoosterNourish™-XF and the culture time and cell yield were compared to competitor cells and media system.

II. EV Yield

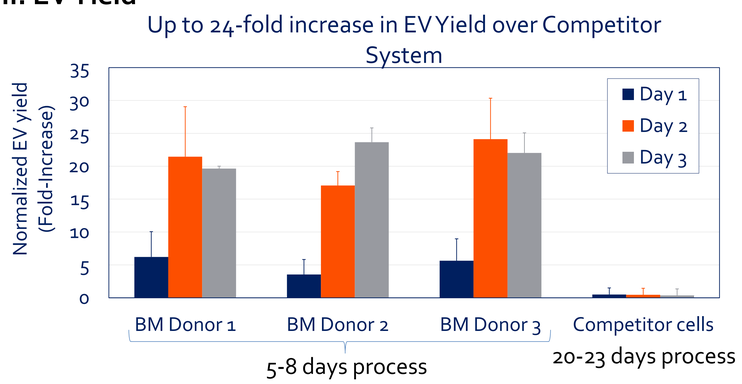


Figure 5: Distinct increase in EV secreted from three different hMSCs donors upon incubation in EV Collection media (day1-3) post expansion.

CASE STUDY 2: ADAPTATION OF A SCALABLE 3D PROCESS FOR MSC-EV COLLECTION

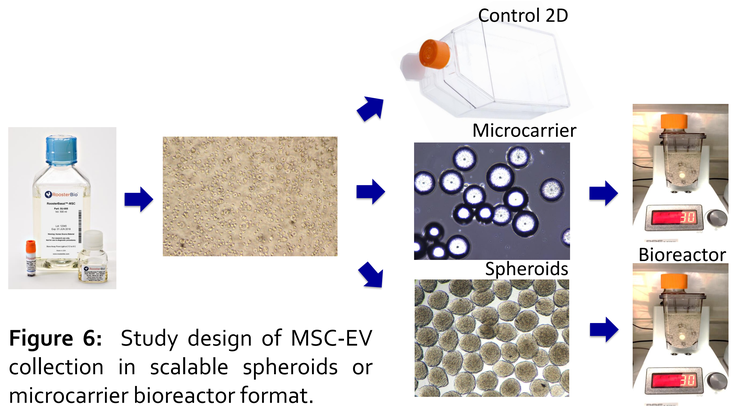


Figure 6: Study design of MSC-EV collection in scalable spheroids or microcarrier bioreactor format.

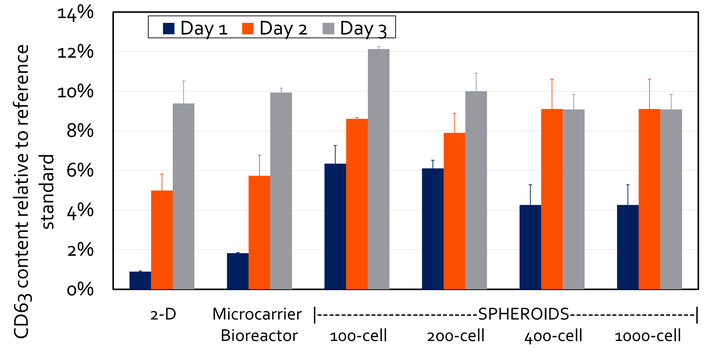


Figure 7: EV yield characterized by CD63 content, was higher in spheroid suspension culture compared to microcarrier bioreactor or 2D cultures, marked by an acute increase within the first day. Differences between various culture formats are less distinct on subsequent days. With increasing size, spheroids produce less EVs, possibly limited by transport barriers impeding EV release from the inner cells.