RoosterBio®
Radically Simplifying Use of MSCs

REVOLUTIONIZING EXTRACELLULAR VESICLE PRODUCTION

EXTRACELLULAR VESICLE (EV) PRODUCTION
HIGH VOLUME hMSCs | BIOPROCESS MEDIA
COLLECTION MEDIA & REAGENTS

A Standardized System Drives Massive Yields. Tunable at Multiple Scales.

Radically shorten the time required to implement extracellular vesicles (EVs) and quickly reach unprecedented production efficiencies. An hMSC-based bioprocess with scalability built in provides a seamless path to bioreactor systems.

Generate significant cell mass, switch to **RoosterCollect™-EV** (collection medium) to collect EVs. Use both RoosterCollect-EV and **EV Boost™** (collection boost reagent) to shrink process time and tune EV generation to your application.

Transition from hMSC expansion to dependable EV collection

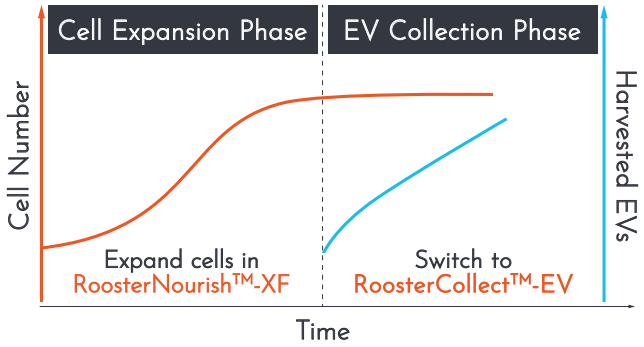


Fig 1(a). Switch from cell expansion phase, using RoosterBio's high volume hMSCs and paired RoosterNourish™-XF bioprocess media, to collection phase using RoosterCollect-EV.

During collection, EV Boost crosses you over to the Boost Zone™

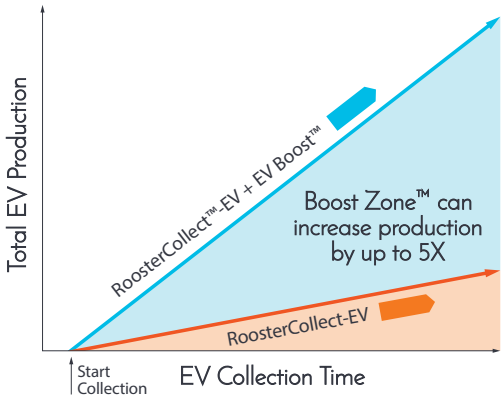


Fig 1(b). Using both RoosterCollect-EV and EV Boost (at high concentrations), 5X particle production and collection can be reached.

A bioprocess engine drives massive EV yields with room to scale

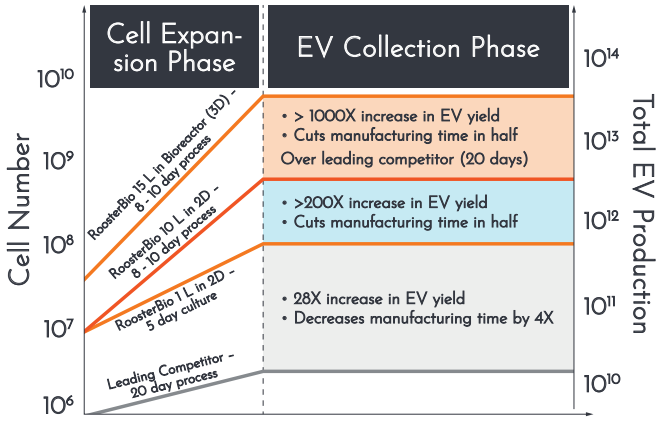
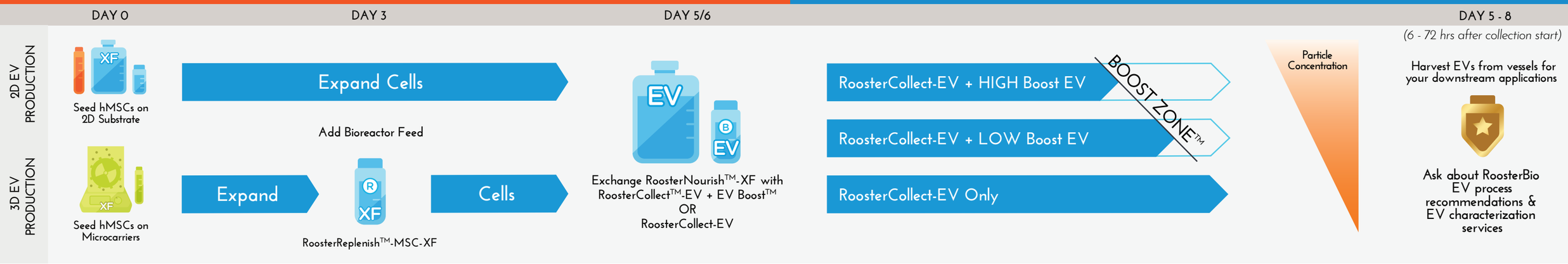


Fig 1(c). Without scale-up, RoosterBio EV Production provides a 28X increase in EV yield and a 4X decrease in manufacturing time over a leading hMSC system. Switching to bioreactor culture, over 1000X increase in EVs can be achieved. When you're ready, RoosterBio EV Production still has room to scale and is tunable to boost efficiencies.

EV Boost stimulates cells to increase EV productivity. Find the Boost concentration that's right for you.

CELL EXPANSION USING ROOSTERBIO XENO-FREE 2D & 3D SYSTEMS

2D & 3D EV COLLECTION USING ROOSTERCOLLECT™-EV W/WO EV BOOST™



ROOSTERCOLLECT-EV SUPPORTS SCALABLE, ULTRA LOW NOISE COLLECTION FROM hMSCs

Reduced particle levels

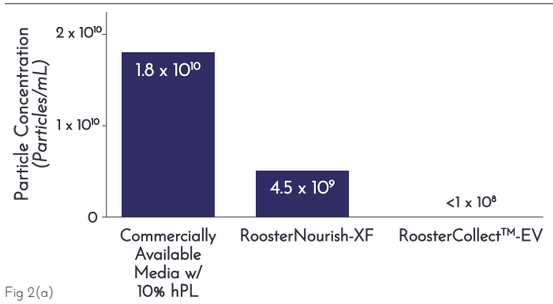


Fig 2(a)

Scalable EV collection in multiple bioreactor sizes

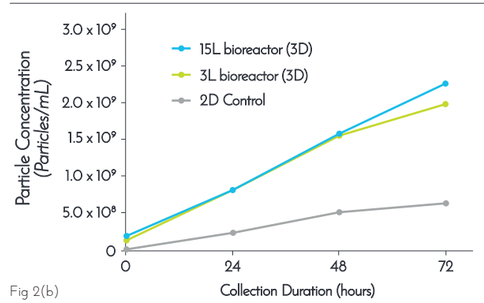


Fig 2(b)

Fig 2(a). RoosterCollect-EV is a low particle medium engineered for extracellular vesicle collection and (b) supports scalable EV collection from 6 to 72 hours in both 2D vessel and 3D bioreactor systems.

CHARACTERIZATION OF ROOSTERBIO XENO-FREE (XF) hMSC-DERIVED EVs

hMSC-derived high quality EVs are consistent with size, protein composition, and RNA content as recommended by ISEV*

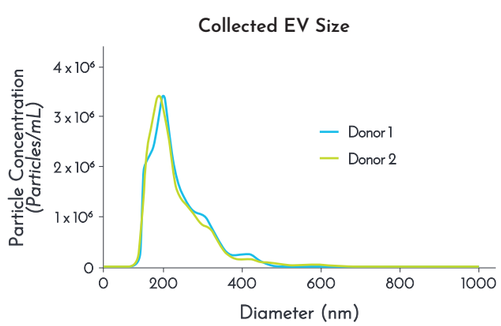


Fig 3(a)

EV TEM Image

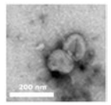


Fig 3(b)

Membrane Protein Immunofluorescence

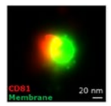


Fig 3(c)

RNA content of RoosterBio hMSC-EVs

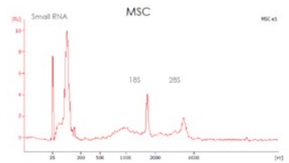


Fig 3(d)

Western Blot of RoosterBio hMSC-EV Protein Content

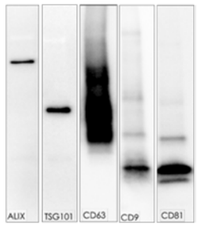


Fig 3(e)

Fig 3(a). Particles collected from RoosterBio xeno-free hMSCs have diameters in the size range of 50 to 250 nms. Fig 3(b) Transmission electron microscope image of collected EV. (c) Hallmark EV transmembrane CD81 protein stain demonstrates localization on outer lipid membrane. (ONI Nanoimager; Oxford Nanoimaging Ltd, Oxford, UK) (d) Collected EVs contain primarily small RNA. (e) Western Blot shows EVs express expected proteins: ALIX, TSG101, CD63, CD9, and CD81.

*Lötvall, J., et al (2014). Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. *Journal of extracellular vesicles, 3, 26913.*

A SYSTEM WITH ROOM TO SCALE, EV BOOST INCREASES EV PRODUCTION AND REDUCES PROCESSING TIME

EV Boost is an optional reagent added to RoosterCollect-EV during EV Collection Phase

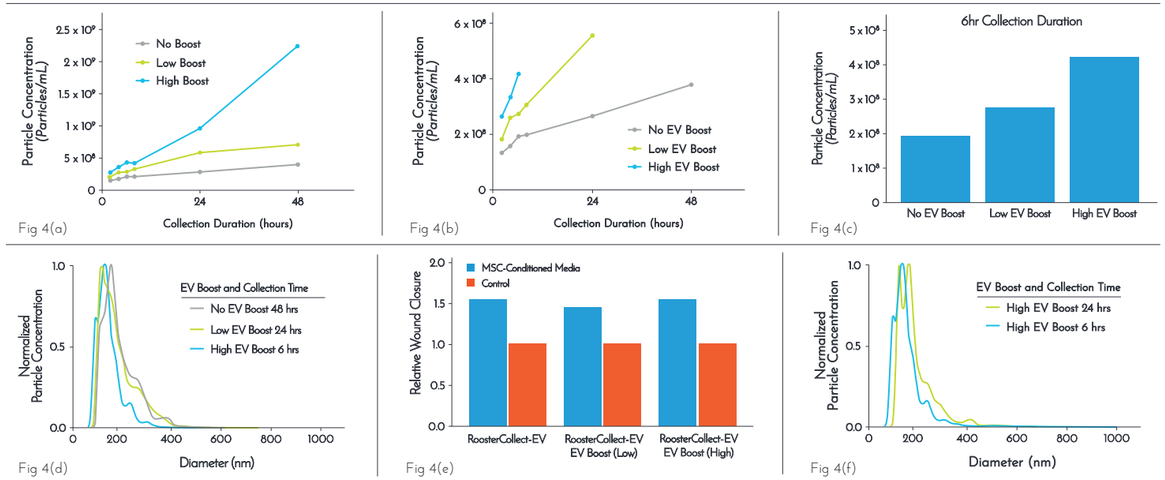
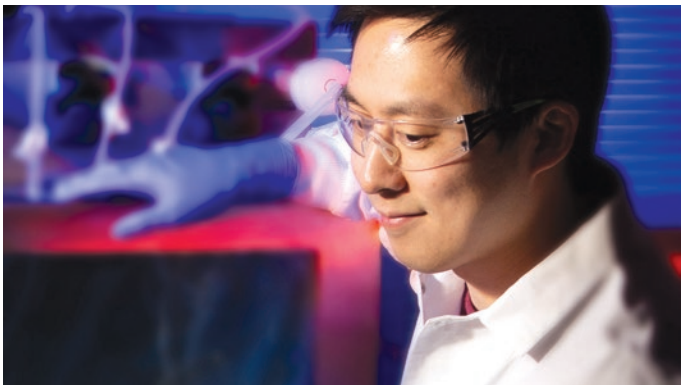


Fig 4(a). EV Boost reagent, as part of the RoosterBio EV Production process can be used to increase particle yield up to 5 fold over a 48 hour collection period. (b) High EV Boost produces similar EV yield at reduced collection times, such as (c) at a 6 hour collection timepoint. (d) EVs collected from hMSCs at shortened High EV Boost times have similar size profiles and (e) bioactivity in a standard in vitro wound healing scratch test as EVs collected from extended Low and No EV Boost collection windows. (f) High EV Boost concentrations at early and late timepoints.

ADVANTAGES OF THE ROOSTERBIO EV PRODUCTION

- **Start with the right materials:** MSC-derived EVs are the gold standard for extracellular factor use in regenerative medicine and cell therapy. Our XF hMSCs and paired bioprocess media are up to 10X more productive (M cells/L) and have industry leading functional characterization – enabling your EV process to begin quickly and with confidence in your critical starting material. You can generate 1B hMSCs in 10 days for $10K (USD).
- **Simple to implement:** Whether you are new to hMSC EVs or moving to increased EV productivity, easy-to-follow streamlined hMSC expansion and EV collection protocols are included with each cell and media shipment, saving you months of process development time and easing workflow implementation.
- **Scales with you:** RoosterCollect-EV and EV Boost seamlessly integrate with our 2D or bioreactor hMSC culture systems as part of a direct hMSC expansion to EV collection workflow that is right sized for your product development needs.
- **Reduced barrier to clinical translation:** Manufactured with the end in mind – RoosterBio XF hMSCs, paired bioprocess media, and EV Production media and reagents comprise a translation-friendly process that reduces the barrier to clinical EV workflow implementation.

Imagine All the EVs You'll Collect.



GENERATING LOTS OF EXTRACELLULAR VESICLES (EVs) STARTS WITH A SCALABLE BIOPROCESS SYSTEM.

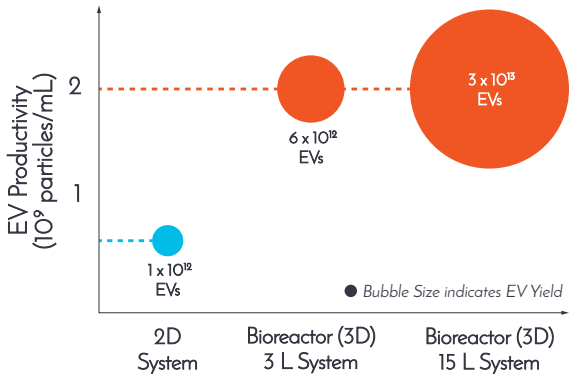


Fig 5. Using a scalable biomanufacturing process, there's a 2X increase in EV productivity between 2D and bioreactor (3D) systems. Bubble size indicates total EV yield.

RoosterBio provides clinical translation-friendly, xeno-free (XF) hMSCs and paired media to quickly and efficiently generate essential cellular materials.

A golden standard for EVs, RoosterBio's hMSC-geared process

> Simplifies implementing EVs into your workflow

> Boosts EV yield

> Minimizes processing time

> Streamlines manufacturing

> Radically increases EV productivity

Catalyze EV Production with RoosterCollect™-EV and EV Boost™, two components engineered and designed to be used together and to supplement RoosterBio's Development Grade products. Up to 5X particle collection can be achieved in the Boost Zone™ using EV Boost.

AREAS OF APPLICATION

Cell Therapy | Regenerative Medicine | Tissue Engineering | Stem Cell-Derived Materials

Catalog No.	Product	Qty	Unit Size & Brief Description
EXTRACELLULAR VESICLE (EV) PRODUCTION			
M2001	**RoosterCollect™-EV**	1 bottle	500 mL collection medium
S2001	**EV Boost™**	1 bottle	30 mL collection boost reagent

To view ordering information for all XF Development Grade products – visit **www.roosterbio.com.**

Learn more at **bit.ly/RoosterBioEVProduction**.

Contact us at **info@roosterbio.com** for batch (2D) and fed-batch (3D) process recommendations.

SEAMLESSLY TRANSITION FROM RAPID CELL EXPANSION TO DEPENDABLE COLLECTION.
EV Production is now tunable to your application.

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