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Media Optimization Panel for MSC Extracellular Vesicle Production

Application note

INTRODUCTION

Extracellular vesicles (EVs) derived from mesenchymal stem/stromal cells (MSCs) are known to induce a biological response such as wound healing, angiogenesis, and immunosuppression. Cell-free therapies, such as those utilizing MSC EVs are being explored extensively. Here, we present a new media panel optimized for MSC extracellular vesicle production. The media panel is composed of 8 different EV Production Media formulations (A-H) and our data showcases the effect of media formulation on MSC extracellular vesicle production as compared to the benchmark EV production medium (B.M.). MSC growth and EVs produced with the different media were characterized, and show better results with media A-H compared to the B.M.

MATERIALS & METHODS

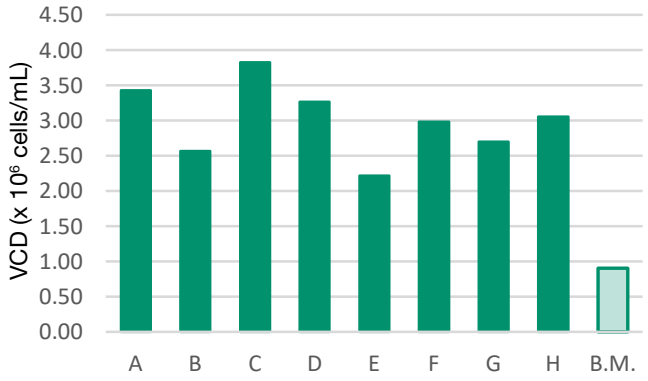
BM-MSCs (ATCC) were seeded into CellBIND T-150 Flask (CORNING) and expanded to approximately 80% confluency using PRIME-XV MSC Expansion XSFM (FUJIFILM Irvine Scientific, Catalog #91149). After washing the cells, the culture media was switched to proprietary EV production panel media or a benchmark and MSCs were cultured for an additional 96 hours. Cells were collected and characterized using a ViCELL XR Cell Analyzer and FACSymphony A3. Culture supernatant was centrifuged at 300 xg for 3 minutes, followed by centrifugation at 2,000 xg for 20 minutes. Collected centrifuged culture supernatant was concentrated using Vivaspin 20 centrifugal concentrator (Sartorius) and EVs were isolated using MagCapture Exosome Isolation Kit PS Ver.2 (FUJIFILM Wako Pure Chemical, Catalog #290-84103). Isolated EVs were analyzed with nCS2 Particle Analyzer and C-400 cartridge (Spectradyne). For further EV characterization, ONI Nanoimager S and EV Profiler Kit (ONI), Tim4-based EV ELISA and Western Blotting were used to characterize EV specific markers.

RESULTS

8 formulations in MSC EV production media panel were evaluated using BM-MSCs in T-150 flask format and commercial EV production media from Supplier A was used as a benchmark. The evaluation resulted in a higher viable cell density (VCD) and viability than the benchmark media **(Figure 1A, B)**. Flow cytometry analysis confirmed MSC surface markers were maintained after the EV production step in both MSC media panel formulations and the benchmark media **(Figure 1C, D)**. MSC EVs were isolated from the supernatant and particle concentration was measured using nCS2 particle counter. All the MSC EV media panel formulations were comparable to or outperformed the benchmark media **(Figure 2A, B)**. EV ELISA and Western Blotting confirmed the isolated EVs contained internal and external EV markers and met MISEV2023 guideline[1] requirements in terms of the presence of EVs features **(Figure 2C, D, E, F)**. Single EV characterization showed a diversity of EV subpopulations in the EV production conditions **(Figure 2G)**.

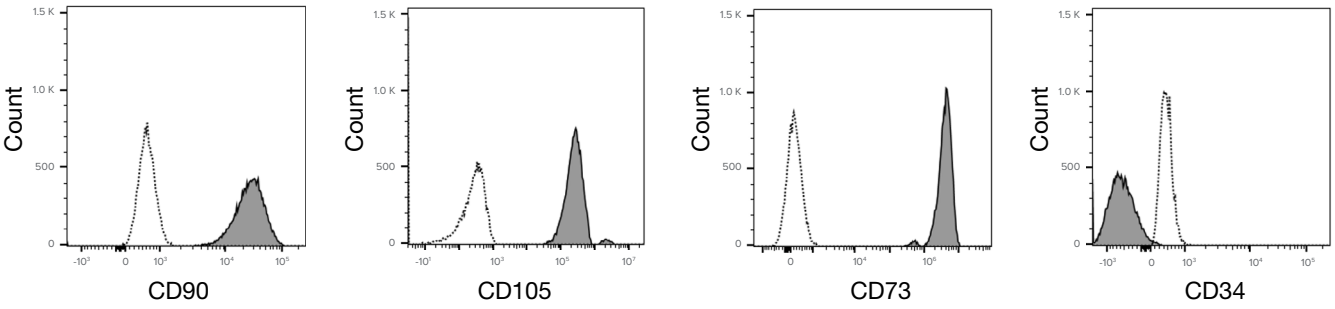
Find the Suitable MSC EV Media for Your Needs

A Viable Cell Density (VCD) After EV Production



B Cell Viability After EV Production

C



D

Media	A	B	C	D	E	F	G	H	B.M.
Positive Markers (%)									
CD90	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CD73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CD105	99.40	99.10	99.60	99.80	99.00	99.80	99.60	99.80	97.00
Negative Markers (%)									
CD34	0.02	0.02	0.04	0.02	0.02	0.02	0.03	0.02	0.21

Figure 1. MSC characterization after EV production. After culture for 96 hours using EV production media panel (A to H) or a benchmark (B.M.) **(A)** Viable cell density (VCD) and **(B)** viability of MSCs after EV production. **(C)(D)** MSC positive and negative marker characterization.

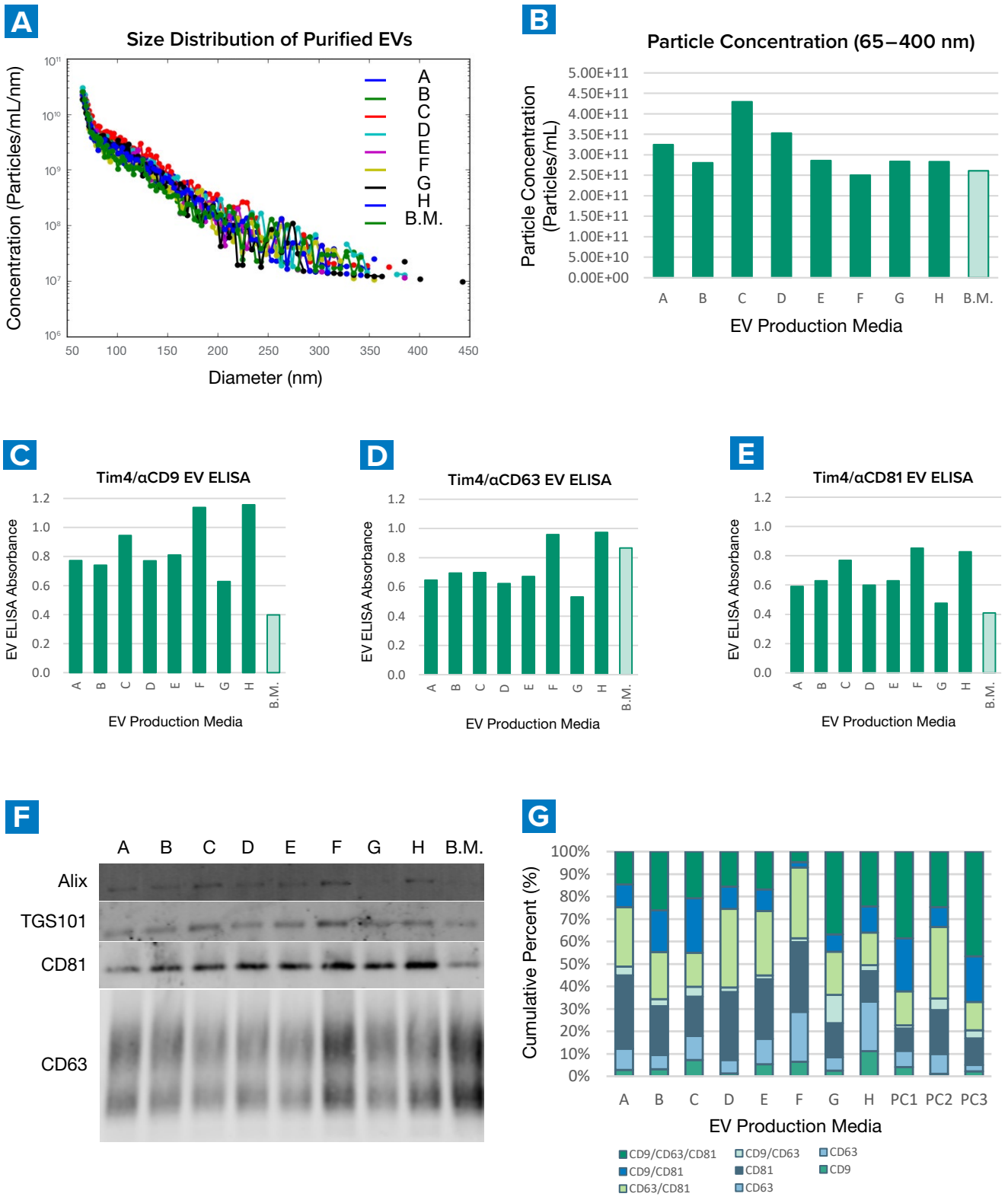


Figure 2. MSC EV Characterization. Conditioned media collected from EV production step was concentrated and the EVs were isolated and analyzed. **(A, B)** Particle size and concentration. **(C, D, E)** EV surface marker characterization using Tim4-based EV ELISA. $1x10^7$ particles/well were used for the input. **(F)** Surface and internal EV marker detection using western blotting. $1x10^9$ particles/lane were used for the input. **(G)** Single EV visualization and characterization using super resolution microscopy. $1x10^9$ particles/lane were used for the input. PC1, PC2, and PC3 are positive control EVs in EV profiler chip 1, chip 2, and chip 3 respectively.

CONCLUSION

MSC EV media panel formulations stimulate MSC EV secretion without disturbing the characteristics of MSCs and affecting the cell viability. EV characterization assays confirmed that the characteristics of EVs meet MISEV2023 guideline[1] in terms of the presence of EVs features. Our media formulations will support high quality and quantity MSC EV production. FUJIFILM Irvine Scientific offers custom media development services that can take any promising media formulation and modify it to meet your particular needs.

References:

1. Welsh, J. A., Goberdhan, D. C., O'Driscoll, L., Buzas, E. I., Blenkiron, C., Bussolati, B. & Beetler, D. J. (2024). Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. Journal of extracellular vesicles, 13(2), e12404.

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