Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supporting Information

Multifaceted effects of Milk-Exosome (Mi-Exo) as a modulator of scar-free wound healing

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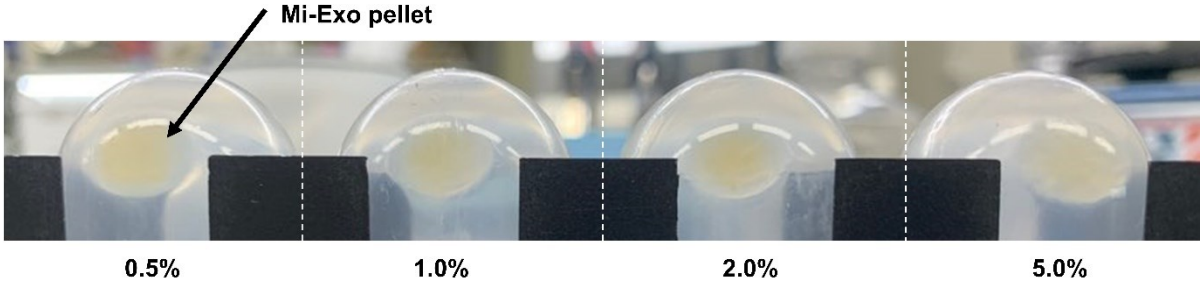

Figure S1. Isolated Mi-Exo. Mi-Exo pellet after ultra-centrifugation. pFtM was treated with range from 0.5 to 5.0 % acetic acid (AA).

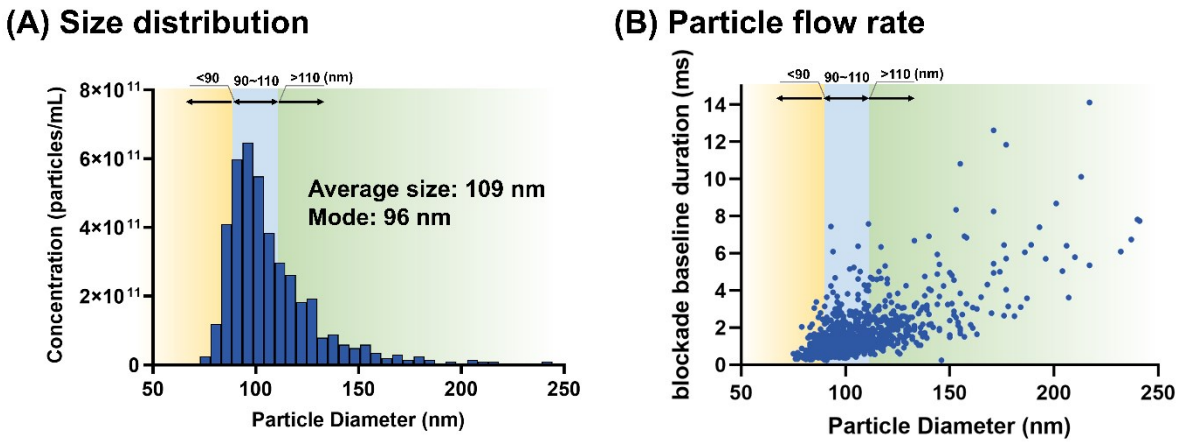

Figure S2. Mi-Exo size properties. (A) Size distribution of Mi-Exo. (B) blockade baseline duration (ms) by particle tracking analysis. Blockade baseline duration means flow rate of Mi-Exo particle.

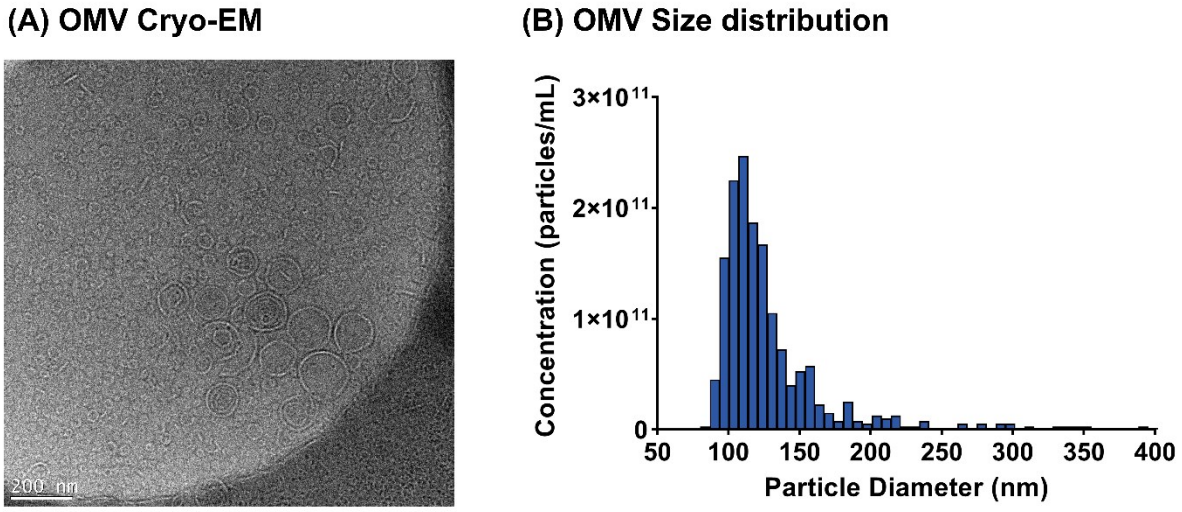

Figure S3. Isolated bacterial OMV characterization. (A) Cryo-EM image of OMV. OMVs also have typical sphere vesicle formed bi-lipid layer structure. (B) Size distribution of OMVs. *E. coli* BL21 (DE3) strain was cultured to 500 mL in 2 L flask (200 rpm, 1 day, 25 ℃). Next, cultured bacteria sample was centrifuged 3,000 rpm, 30 min, 4 ℃, and supernatant filtered by using (0.45 and 0.22) μm bottle-top vacuum filter. Filtered supernatant was centrifuged at 200,000× *g* for 2 h at 4 ℃, and washed with 10 mM DPBS under the same condition. OMV pellet was dispersed in 10 mM PBS buffer, and left overnight at 4 ℃. The final products were used in this study.

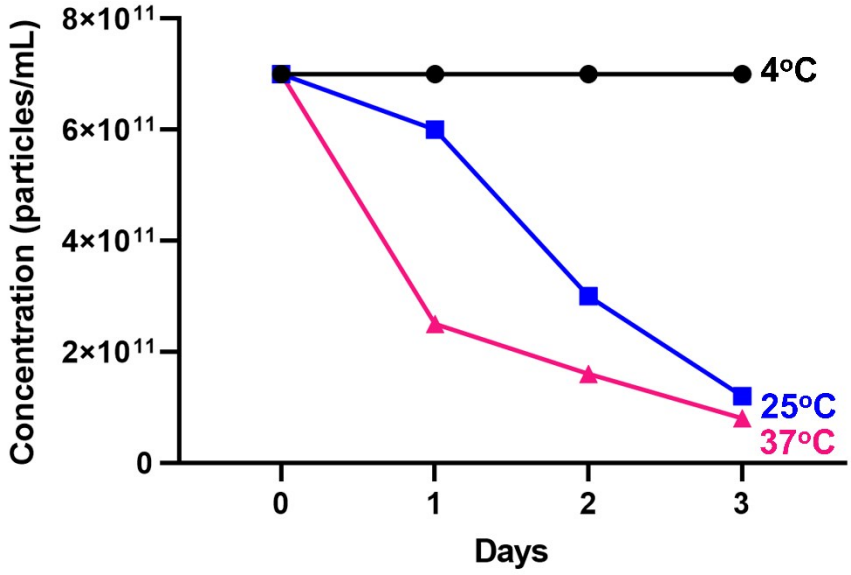

Figure S4. Stability test. Mi-Exo stored at three different temperatures of 4 ℃, 25 ℃, and 37 ℃. There were no significant differences between particle concentrations at 4 °C, after day 3. However, at 25 and 37 °C, there were a significant decrease in the NP Mi-Exo decreasing from 14.3 – 64.3% at day 1 to 82.9 – 88.6% after day 3, respectively. NP: number of particles.

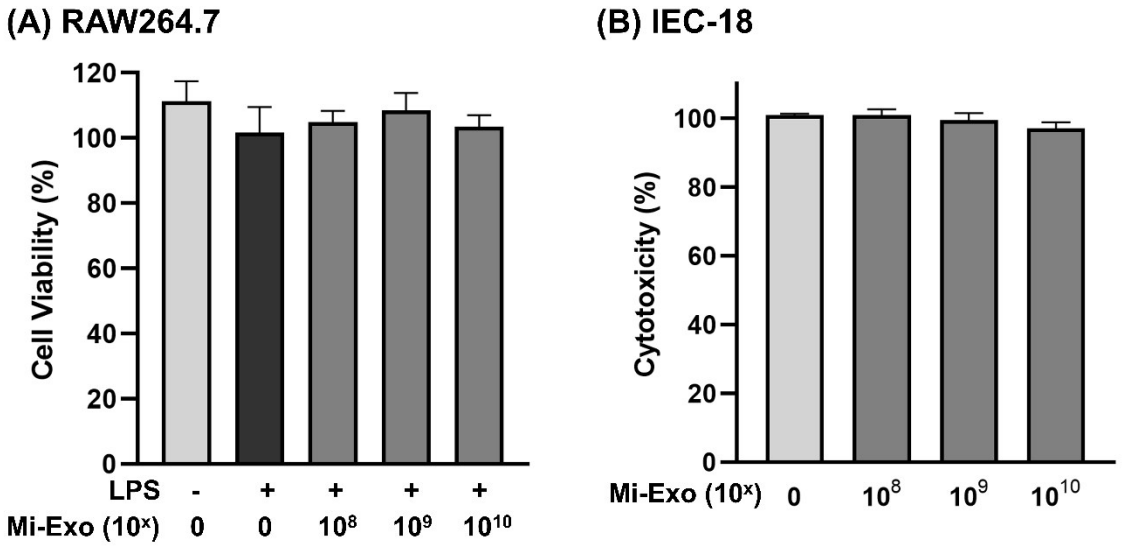

Figure S5. Cell viability of LAW264.7 and IEC-18 cells. (A) Cell viability of RAW264.7 cell. Although LPS treatment showed a tendency to decrease slightly, compared to the untreated group, this did not significantly affect the experiment. (B) Cell viability of the IEC-18 cell. All results were measured in triplicate. For the cytotoxicity of Mi-Exo, RAW264.7 and IEC-18 cells were seeded into 96-well plate (3×10^5 cells/well), and incubated for 24 h. Then, Mi-Exo was added to plate, and further incubated for 24 h. The cells were then washed with DPBS. Cytotoxicity was measured by Cell Counting Kit-8 (Dojindo, Kumamoto, Japan), and the procedure was performed by manual protocol. Reading absorbance used ELISA plate reader.

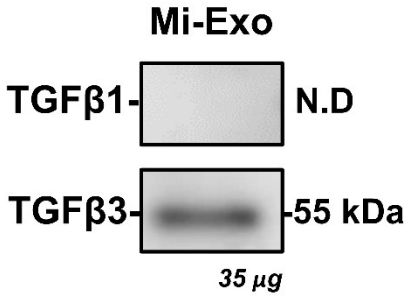

Figure S6. TGFβ1 and TGFβ3 in Mi-Exo. The protein level of TGF-β1 and TGF-β3 in Mi-Exo was analyzed by Western blot. The entire experimental procedure is described in 2.2.

Table S1. MicroRNA primer list used in this study.

Primer	Sequence (5'-3')	Reference
Adaptor primer	GCGAGCACAGAATTAATACGACTCACTATA GGTTTTTTTTTTTTTTTT	[1]
Bta-miR-2478	GTATCCCACTTCTGACACCA	miR Base[a]
Universal primer	GCGAGCACAGAATTAATACGAC	[1]

[a]Bta-miR sequence information can be found at the miR Base web site

(http://www.mirbase.org/).

Table S2. Cytokine primer list used in this study.

Species	Primer		Sequence (5'-3')	Reference
Mouse	TNFα	F	CCACCACGCTCTTCTGTCTAC	2
		R	AGGGTCTGGGCCATAGAACT	
	IL-6	F	GCTACCAAACTGGATATAATCAGGA	3
		R	CCAGGTAGCTATGGTACTCCAGAA	
	iNOS	F	TCTTTGACGCTCGGAACTGT	4
		R	CCATGATGGTCACATTCTGC	
	COX2	F	AGCCCACCCCAAACACAGT	5
		R	AAATATGATCTGGATGTCAGCACATATT	
	Actin	F	GTGGGCCGCTCTAGGCACCAA	6
		R	CTCTTTGATGTCACGCACGATTTC	
	TGFβ1	F	GTGTGGAGCAACATGTGGAACTCTA	7
		R	TTGGTTCAGCCACTGCCGTA	
	TGFβ3	F	GCTCTTCCAGATACTTCGAC	8
		R	AGCAGTTCTCCTCCAGGTTG	

The Mi-Exo was pre-denatured at 95 ℃ for 3 min, followed by 45 cycles of denaturating at 95 ℃ for 15 s, annealing at 60 ℃ for 30 s, and extension at 72 ℃ for 30 s. Table S2 shows the primer list used for mRNA RT-PCR.

Reference

1. D. Zheng, S. Haddadin, Y. Wang, L. Q. Gu, M. C. Perry, C. E. Freter and M. X. Wang, *Int J Clin Exp Pathol*, 2011, **4**, 575-586.
2. K. Ogino, R. Zhang, H. Takahashi, K. Takemoto, M. Kubo, I. Murakami, D. H. Wang and Y. Fujikura, *PLoS One*, 2014, **9**, e92710.
3. M. Zhang, C. Wang, J. Wu, X. Ha, Y. Deng, X. Zhang, J. Wang, K. Chen, J. Feng, J. Zhu, J. Xie and J. Zhang, *Mediators Inflamm*, 2018, **2018**, 1756494.
4. J. S. Hwang, K. H. Kim, J. Park, S. M. Kim, H. Cho, Y. Lee and I. O. Han, *J Biol Chem*, 2019, **294**, 608-622.
5. E. Javorkova, P. Trosan, A. Zajicova, M. Krulova, M. Hajkova and V. Holan, *Stem Cells Dev*, 2014, **23**, 2490-2500.
6. Y. W. Lin, C. Slape, Z. Zhang and P. D. Aplan, *Blood*, 2005, **106**, 287-295.
7. T. Tsumuraya, H. Ohtaki, D. Song, A. Sato, J. Watanabe, Y. Hiraizumi, T. Nakamachi, Z. Xu, K. Dohi, H. Hashimoto, T. Atsumi and S. Shioda, *J Neuroinflammation*, 2015, **12**, 35.
8. T. W. Gilbert, A. M. Stewart-Akers, J. Sydeski, T. D. Nguyen, S. F. Badylak and S. L. Woo, *Tissue Eng*, 2007, **13**, 1313-1323.