

Supplementary Materials

Mechanically remodeled extracellular vesicles: drivers of disease or therapeutic opportunities?

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Supplementary Table 1. Comparison-level methodological details and reported changes in EV output following mechanical stimulation.

Stimulus type	Stimulation mode	EV source	Isolation method	Quantification method	Normalization approach	Stimulated /control	Ref.
direct stimulation	compression	BM-MSCs	Ultracentrifugation	Flow NanoAnalyzer N30E	sEV number/cell	4	[1]
direct stimulation	compression	HU-MSCs	Ultrafiltration; Ultracentrifugation	Nanoparticle tracking analysis	total particle number	4	[2]
direct stimulation	shear stress	C2C12 cells	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	1	[3]
direct stimulation	shear stress	DPSCs	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	37	[4]
direct stimulation	shear stress	HASMCs	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	4.2	[5]
direct stimulation	shear stress	hBM-MSCs	Ultracentrifugation	Nanoparticle tracking analysis	particles/ (5×10^6) cells	10	[6]
direct stimulation	shear stress	HeLa cervical cancer cells	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	2	[7]
direct stimulation	shear stress	Human red blood cells	Ultracentrifugation; exoEasy Maxi membrane-affinity column	Tunable resistive pulse sensing	Concentration was normalized to the starting supernatant volume	6.6	[8]
direct stimulation	shear stress	HUVECs	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	1	[9]

direct stimulation	shear stress	HUVECs	Ultracentrifugation; Size-Exclusion Chromatography	Tunable resistive pulse sensing	particles/mL	5	[10]
direct stimulation	shear stress	MDA-MB-231 breast cancer cells	Ultracentrifugation	Nanoparticle tracking analysis	the number per 10 ⁶ cells	3	[11]
direct stimulation	shear stress	MLO-Y4	Ultrafiltration; ExoQuick-TC	Nanoparticle tracking analysis	Not reporte	6.2	[12]
direct stimulation	shear stress	MSCs	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	40	[4]
direct stimulation	stretch	HEK293T	Ultraultrafiltration; Tangential Flow Filtration	Nanoparticle tracking analysis	particles per cell ($\times 10^4$)	9.8	[13]
direct stimulation	stretch	HMEC-1	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	2.5	[14]
direct stimulation	stretch	MLE-12 cell	Ultracentrifugation	Nanoparticle tracking analysis	normalized to the number of live cells	2	[15]
direct stimulation	stretch	SkMCs	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	11	[4]
direct stimulation	ultrasound	A549 Cells	Exoquick-TC	Nanoparticle tracking analysis	particles/cell	2.5	[16]
direct stimulation	ultrasound	ADSCs	Ultrafiltration	Nanoparticle tracking analysis	fold change relative to unstimulated controls.	45	[17]
direct stimulation	ultrasound	ADSCs	Ultracentrifugation	Nanoparticle tracking analysis	relative particle concentration	1.5	[18]

direct stimulation	ultrasound	BMSC	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	3.66	[19]
direct stimulation	ultrasound	BMSCs	Ultracentrifugation	Nanoparticle tracking analysis	Not reporte	1	[20]
direct stimulation	ultrasound	C2C12 myoblasts	Ultracentrifugation	Tunable resistive pulse sensing	particles/mL	1.4	[21]
direct stimulation	ultrasound	C2C12 myoblasts	Ultracentrifugation	Tunable resistive pulse sensing	particles/mL	3	[21]
direct stimulation	ultrasound	C2C12 myotubes	Exo Quick-TC	Tunable resistive pulse sensing	particles/mL	2	[22]
direct stimulation	ultrasound	C2C12 myotubes	Differential ultracentrifugation	Nanoparticle tracking analysis	particles/mL	2	[23]
direct stimulation	ultrasound	chondrocytes	Ultrafiltration	Nanoparticle tracking analysis	fold change relative to control	16	[24]
direct stimulation	ultrasound	Human astrocytes	Exoquick-TC	Nanoparticle tracking analysis	particles/mL	4.14	[25]
direct stimulation	ultrasound	Human pharyngeal squamous carcinoma cells	ExoCap magnetic capture beads CD9 and CD63 from JSR Life Sciences	shown by the CD9 and CD63 fluorescent intensities of EVs	CD9 and CD63 fluorescent intensities of EVs	4	[26]
direct stimulation	ultrasound	PDLSCs	Polymer precipitation	Nanoparticle tracking analysis	particles/mL	2	[27]
direct stimulation	ultrasound	RAW 264.7	Ultrafiltration	Nanoparticle tracking analysis	particles/mL	2	[28]

direct stimulation	ultrasound	Schwann Cells	Invitrogen Total Exosome Isolation Reagent	exosome protein concentration	Not reporte	3	[29]
direct stimulation	ultrasound	U87-MG	PureExo® Exosome Isolation Kit	Nanoparticle tracking analysis	particles/mL	10	[30]
indirect stimulation	3D culture	hUCMSCs	Tangential flow filtration	ZetaView PMX-120 in 488-nm scatter mode	particles/cell	6.7	[31]
indirect stimulation	3D culture	hucMSCs	Ultracentrifugation	BCA protein assay	EV-associated protein	19.4	[32]
indirect stimulation	3D culture	U-MSC	Tangential flow filtration	Nanoparticle tracking analysis	the number of EVs divided by the number of cells	20	[33]
indirect stimulation	osmotic stress	APCs	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	7.5	[34]
indirect stimulation	osmotic stress	APCs	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	8	[34]
indirect stimulation	osmotic stress	HEK293T	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	3	[35]
indirect stimulation	stiffness	BMDMs	Precipitation-based exosome extraction kit	Nanoparticle tracking analysis	particles/mL	5	[36]
indirect stimulation	stiffness	mouse cardiac fibroblasts	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	1	[37]
indirect stimulation	stiffness	mouse mammary fibroblasts	Ultracentrifugation	Nanoparticle tracking analysis	particles/mL	1	[37]
indirect stimulation	stiffness	mPDAC	Ultracentrifugation	flow cytometer with a small-particle option	total EVs/mL	1	[38]

mixed stimulation	mixed stimulation	macrophage	Ultracentrifugation	Nanoparticle tracking analysis	total NTA particle count/total cell count	12.5	[39]
mixed stimulation	mixed stimulation	AT-MSCs	the Total Exosome Isolation reagent	Nanoparticle tracking analysis	particles/cell	3.7	[40]
mixed stimulation	mixed stimulation	BM-MSCs	the Total Exosome Isolation reagent	Nanoparticle tracking analysis	particles/cell	1.4	[40]
mixed stimulation	mixed stimulation	BM-hMSCs	Ultrafiltration; Ultracentrifugation; 16% PEG/1 M NaCl	Nanoparticle tracking analysis	EVs/10 ⁶ cells/day	2.5	[41]
mixed stimulation	mixed stimulation	BMSCs	Ultracentrifugation	Nanoparticle tracking analysis	total NTA particle count/total cell count	18.2	[39]
mixed stimulation	mixed stimulation	choroid plexus (ChP)	ExtraPEG-based differential ultracentrifugation	Nanoparticle tracking analysis	particles/mL.	4	[42]
mixed stimulation	mixed stimulation	HEK293T	Ultraultrafiltration; Tangential Flow Filtration	Nanoparticle tracking analysis	particles per cell ($\times 10^4$)	115	[13]
mixed stimulation	mixed stimulation	hiPSCs (TC-1133)	ExtraPEG-based ultracentrifugation	Nanoparticle tracking analysis	total particle number/(50 mL spent medium)	23	[43]
mixed stimulation	mixed stimulation	hMSCs	ExtraPEG precipitation combined with ultracentrifugation	Nanoparticle tracking analysis	EVs/cell/2 days	2	[44]
mixed stimulation	mixed stimulation	human blood vessel organoids	ExtraPEG precipitation combined with ultracentrifugation	Nanoparticle tracking analysis	normalizing EV numbers to spent media and cell number	3	[45]

mixed stimulation	mixed stimulation	HUVECs	Ultracentrifugation	Nanoparticle tracking analysis	total NTA particle count/total cell count	9.4	[39]
mixed stimulation	mixed stimulation	NK-92MI cell	Ultracentrifugation	Nanoparticle tracking analysis	particles/cell/day	1.91	[46]
mixed stimulation	mixed stimulation	Schwann cells	Ultracentrifugation	Nanoparticle tracking analysis	particles per T75 flask; 1.5 × 10 ⁶ cells and 10 mL medium per flask	1	[47]
mixed stimulation	mixed stimulation	UCM-MSCs	the Total Exosome Isolation reagent	Nanoparticle tracking analysis	particles/cell	3.9	[40]
mixed stimulation	mixed stimulation	UC-MSCs	Ultracentrifugation	Bradford assay	exosomal protein (μg)/original conditioned medium (mL)	7.5	[48]
mixed stimulation	mixed stimulation	WJ-MSCs	Ultracentrifugation followed by OptiPrep iodixanol density-gradient ultracentrifugation	Nanoparticle tracking analysis	particles/cell	6	[49]

REFERENCE

1. Hao R, Hu S, Zhang H, Chen X, Yu Z, et al. Mechanical stimulation on a microfluidic device to highly enhance small extracellular vesicle secretion of mesenchymal stem cells. *Materials Today Bio.* 2023;18:100527. [DOI: 10.1016/j.mtbio.2022.100527]
2. Bai C, Ren H, Zhang F, Li D, Feng Q, et al. Normal stress on surface of mesenchymal stem cells boosts extracellular vesicle secretion and regenerative bioactivity. *Journal of Nanobiotechnology.* 2025;23:[DOI: 10.1186/s12951-025-03556-y]
3. Takafuji Y, Tatsumi K, Kawao N, Okada K, Muratani M, et al. Effects of fluid flow shear stress to mouse muscle cells on the bone actions of muscle cell-derived extracellular vesicles. *PLOS ONE.* 2021;16:e0250741. [DOI: 10.1371/journal.pone.0250741]
4. Guo S, Debbi L, Zohar B, Samuel R, Arzi R, et al. Stimulating extracellular vesicles production from engineered tissues by mechanical forces. *Nano Lett.* 2021;21:2497-504. [DOI: 10.1021/acs.nanolett.0c04834]
5. Gao W, Gu K, Ma L, Yang F, Deng L, et al. Interstitial fluid shear stress induces the synthetic phenotype switching of VSMCs to release pro-calcified extracellular vesicles via EGFR-MAPK-KLF5 pathway. *International Journal of Biological Sciences.* 2024;20:2727-47. [DOI: 10.7150/ijbs.90725]
6. Seo Y, Kang H, Park J. Shear stress-induced influx of extracellular calcium ions: a pivotal trigger amplifying the production of mesenchymal stem cell-derived extracellular vesicles. *Nanoscale.* 2025;17:3861-72. [DOI: 10.1039/d4nr01215a]
7. Wang K, Wei Y, Liu W, Liu L, Guo Z, et al. Mechanical stress-dependent autophagy component release via extracellular nanovesicles in tumor cells. *ACS Nano.* 2019;13:4589-602. [DOI: 10.1021/acsnano.9b00587]
8. Sangha GS, Weber CM, Sapp RM, Setua S, Thangaraju K, et al. Mechanical stimuli such as shear stress and piezo1 stimulation generate red blood cell extracellular vesicles. *Front Physiol.* 2023;14:1246910. [DOI: 10.3389/fphys.2023.1246910]
9. Kardani A, Hemmer J, Diesel B, Mashayekhi V, Schomisch A, et al. Laminar flow alters EV composition in HUVECs: a study of culture medium optimization and molecular profiling of vesicle cargo. *Small Methods.* 2025;10.1002/smt.202401841:[DOI: 10.1002/smt.202401841]
10. Coly P, Chatterjee S, Mezine F, Jekmek C, Devue C, et al. Low fluid shear stress stimulates the uptake of noxious endothelial extracellular vesicles via MCAM and PECAM-1 cell adhesion molecules. *J Extracell Vesicles.* 2024;13:e12414. [DOI: 10.1002/jev.2.12414]

11. Brown S, Radcliffe M, Danner J, Cruz W, Lackey K, et al. Extracellular vesicle-mediated modulation of stem-like phenotype in breast cancer cells under fluid shear stress. *Biomolecules*. 2024;14:10.3390/biom14070757. [DOI: 10.3390/biom14070757]
12. Lee S, Jung D, Im S, You C, Kim J, et al. Ank-mediated pyrophosphate regulates shear stress-induced small extracellular vesicle production in 3d-cultured osteocytes. *Anim Cells Syst (Seoul)*. 2024;28:495-505. [DOI: 10.1080/19768354.2024.2409460]
13. Chen Y, Lin Y, Ho C, Chen C, Yu M, et al. High-yield extracellular vesicle production from HEK293t cells encapsulated in 3d auxetic scaffolds with cyclic mechanical stimulation for effective drug carrier systems. *Biofabrication*. 2024;16:10.1088/758-5090/ad728b. [DOI: 10.1088/1758-5090/ad728b]
14. Qiu Y, Lv Z, Zhu X, Yang X, Wan J. 3d mechanical stimulation modulates endothelial exosomes to promote fibroblast activation. *Tissue Cell*. 2026;99:103316. [DOI: 10.1016/j.tice.2026.103316]
15. Najrana T, Mahadeo A, Eid R, Kreienberg E, Schulte V, et al. Mechanical stretch regulates the expression of specific miRNA in extracellular vesicles released from lung epithelial cells. *J Cell Physiol*. 2020;235:8210-23. [DOI: 10.1002/jcp.29476]
16. Lei Z, Jiang H, Liu J, Liu Y, Wu D, et al. Audible Acoustic Wave Promotes EV Formation and Secretion from Adherent Cancer Cells via Mechanical Stimulation. *ACS Applied Materials & Interfaces*. 2023;15:53859-70. [DOI: 10.1021/acsami.3c13845]
17. Zheng Y, Xu P, Pan C, Wang Y, Liu Z, et al. Production and Biological Effects of Extracellular Vesicles from Adipose-Derived Stem Cells Were Markedly Increased by Low-Intensity Ultrasound Stimulation for Promoting Diabetic Wound Healing. *Stem Cell Reviews and Reports*. 2022;19:784-806. [DOI: 10.1007/s12015-022-10487-w]
18. Jiang R, Wang H, Zhong F, Hu Y, Liu J, et al. Low-intensity pulsed ultrasound as a strategy to boost exosome secretion by adipose-derived stem cells and uptake for myocardial infarction therapy. *Biomedical Materials*. 2025;20:045025. [DOI: 10.1088/1748-605x/ade925]
19. Li X, Zhong Y, Zhou W, Song Y, Li W, et al. Low-intensity pulsed ultrasound (LIPUS) enhances the anti-inflammatory effects of bone marrow mesenchymal stem cells (BMSCs)-derived extracellular vesicles. *Cellular & Molecular Biology Letters*. 2023;28:[DOI: 10.1186/s11658-023-00422-3]
20. Wu B, Zhang T, Chen H, Shi X, Guan C, et al. Exosomes derived from bone marrow mesenchymal stem cell preconditioned by low-intensity pulsed ultrasound stimulation promote bone-tendon interface fibrocartilage regeneration and ameliorate rotator cuff fatty

- infiltration. *Journal of Orthopaedic Translation*. 2024;48:89-106. [DOI: 10.1016/j.jot.2024.07.009]
21. Ma X, Yamaguchi A, Maeshige N, Tanida K, Uemura M, et al. Facilitatory effect of low-pulse repetition frequency ultrasound on release of extracellular vesicles from cultured myotubes. *Journal of Medical Ultrasonics*. 2024;51:397-405. [DOI: 10.1007/s10396-024-01429-9]
 22. Yamaguchi A, Maeshige N, Noguchi H, Yan J, Ma X, et al. Pulsed ultrasound promotes secretion of anti-inflammatory extracellular vesicles from skeletal myotubes via elevation of intracellular calcium level. *eLife*. 2023;12:[DOI: 10.7554/elife.89512]
 23. Maeshige N, Langston PK, Yuan Z-M, Kondo H, Fujino H. High-intensity ultrasound irradiation promotes the release of extracellular vesicles from C2C12 myotubes. *Ultrasonics*. 2021;110:106243. [DOI: 10.1016/j.ultras.2020.106243]
 24. Wang Y, Liu Z, Pan C, Zheng Y, Chen Y, et al. Ultrasound-Driven Healing: Unleashing the Potential of Chondrocyte-Derived Extracellular Vesicles for Chondrogenesis in Adipose-Derived Stem Cells. *Biomedicines*. 2023;11:2836. [DOI: 10.3390/biomedicines11102836]
 25. Deng Z, Wang J, Xiao Y, Li F, Niu L, et al. Ultrasound-mediated augmented exosome release from astrocytes alleviates amyloid- β -induced neurotoxicity. *Theranostics*. 2021;11:4351-62. [DOI: 10.7150/thno.52436]
 26. Yuana Y, Jiang L, Lammertink B, Vader P, Deckers R, et al. Microbubbles-Assisted Ultrasound Triggers the Release of Extracellular Vesicles. *International Journal of Molecular Sciences*. 2017;18:1610. [DOI: 10.3390/ijms18081610]
 27. Xiang M, Guo Q, Liu Y, Zhang G, Liao C, et al. Low-intensity pulsed ultrasound enhances the osteogenic potential of PDLSCs-derived extracellular vesicles through COMP/PI3k/AKT. *Faseb J*. 2025;39:10.1096/fj.202402463r. [DOI: 10.1096/fj.202402463r]
 28. Li W, Saleh NA, Gao C, Gagea MA, Vitija X, et al. Dynamic reorganization of multivesicular bodies and exosome production impacted by sonoporation. *Scientific Reports*. 2024;14:[DOI: 10.1038/s41598-024-79042-6]
 29. Peng D, Reed-Maldonado A, Zhou F, Tan Y, Yuan H, et al. Exosome released from schwann cells may be involved in microenergy acoustic pulse-associated cavernous nerve regeneration. *J Sex Med*. 2020;17:1618-28. [DOI: 10.1016/j.jsxm.2020.05.018]
 30. Ambattu L, Ramesan S, Dekiwadia C, Hanssen E, Li H, et al. High frequency acoustic cell stimulation promotes exosome generation regulated by a calcium-dependent mechanism. *Commun Biol*. 2020;3:10.1038/s42003-020-01277-6. [DOI: 10.1038/s42003-020-01277-6]

31. Kim J, Rhim W, Cha S, Woo J, Lee J, et al. Bolstering the secretion and bioactivities of umbilical cord MSC-derived extracellular vesicles with 3d culture and priming in chemically defined media. *Nano Conver.* 2022;9:10.1186/s40580-022-00349-z. [DOI: 10.1186/s40580-022-00349-z]
32. Cao J, Wang B, Tang T, Lv L, Ding Z, et al. Three-dimensional culture of MSCs produces exosomes with improved yield and enhanced therapeutic efficacy for cisplatin-induced acute kidney injury. *Stem Cell Res Ther.* 2020;11:10.1186/s13287-020-01719-2. [DOI: 10.1186/s13287-020-01719-2]
33. Haraszti R, Miller R, Stoppato M, Sere Y, Coles A, et al. Exosomes produced from 3d cultures of MSCs by tangential flow filtration show higher yield and improved activity. *Mol Ther.* 2018;26:2838-47. [DOI: 10.1016/j.ymthe.2018.09.015]
34. Parvanian S, Zha H, Su D, Xi L, Jiu Y, et al. Exosomal vimentin from adipocyte progenitors protects fibroblasts against osmotic stress and inhibits apoptosis to enhance wound healing. *International Journal of Molecular Sciences.* 2021;22:4678. [DOI: 10.3390/ijms22094678]
35. Pironti G, Strachan R, Abraham D, Mon-Wei Yu S, Chen M, et al. Circulating exosomes induced by cardiac pressure overload contain functional angiotensin II type 1 receptors. *Circulation.* 2015;131:2120-30. [DOI: 10.1161/CIRCULATIONAHA.115.015687]
36. Yang Z, Li R, An L, Zhang X, Cheng J, et al. Tumor Mechanobiology-Inspired Nanomechanics: Piezo1 Mediated Tumor-Associated Macrophages Reprogramming and Exosome-Driven Immune Amplification. *ACS Nano.* 2025;19:41746-64. [DOI: 10.1021/acsnano.5c14785]
37. Yang J, Celebi L, Hawthorne L, Ozcebe S, Zorlutuna P. Substrate stiffness modulates fibroblast extracellular vesicle secretion via mechanotransduction pathways. *Small.* 2025;10.1002/smll.202505261. [DOI: 10.1002/smll.202505261]
38. Ferrara B, Bourgoin-Voillard S, Habert D, Vallée B, Nicolas-Boluda A, et al. Matrix stiffness regulates the protein profile of extracellular vesicles of pancreatic cancer cell lines. *Proteomics.* 2024;e2400058. [DOI: 10.1002/pmic.202400058]
39. Huang H, Xiao L, Fang L, Lei M, Liu Z, et al. Static topographical cue combined with dynamic fluid stimulation enhances the macrophage extracellular vesicle yield and therapeutic potential for bone defects. *ACS Nano.* 2025;10.1021/acsnano.4c15201. [DOI: 10.1021/acsnano.4c15201]
40. de Almeida Fuzeta M, Bernardes N, Oliveira F, Costa A, Fernandes-Platzgummer A, et al. Scalable production of human mesenchymal stromal cell-derived extracellular vesicles under

serum-/xeno-free conditions in a microcarrier-based bioreactor culture system. *Front Cell Dev Biol.* 2020;8:10.3389/fcell.2020.553444]. [DOI: 10.3389/fcell.2020.553444]

41. Jeske R, Liu C, Duke L, Castro M, Muok L, et al. Upscaling human mesenchymal stromal cell production in a novel vertical-wheel bioreactor enhances extracellular vesicle secretion and cargo profile. *Bioact Mater.* 2023;25:732-47. [DOI: 10.1016/j.bioactmat.2022.07.004]
42. Ene J, Muok L, Gonzalez V, Sanchez N, Nathani A, et al. Biomanufacturing and curcumin-loading of human choroid plexus organoid-derived extracellular vesicles from a vertical-wheel bioreactor to alleviate neuro-inflammation. *Biomedicines.* 2025;13:1069. [DOI: 10.3390/biomedicines13051069]
43. Muok L, Sun L, Esmonde C, Worden H, Vied C, et al. Extracellular vesicle biogenesis of three-dimensional human pluripotent stem cells in a novel vertical-wheel bioreactor. *Journal of Extracellular Biology.* 2024;3:10.1002/jex2.133]. [DOI: 10.1002/jex2.133]
44. Yuan X, Sun L, Jeske R, Nkosi D, York S, et al. Engineering extracellular vesicles by three-dimensional dynamic culture of human mesenchymal stem cells. *J Extracell Vesicles.* 2022;11:10.1002/jev2.12235]. [DOI: 10.1002/jev2.12235]
45. Ene J, Liu C, Syed F, Sun L, Berry D, et al. Biomanufacturing and lipidomics analysis of extracellular vesicles secreted by human blood vessel organoids in a vertical wheel bioreactor. *Stem Cell Res Ther.* 2025;16:10.1186/s13287-025-04317-2]. [DOI: 10.1186/s13287-025-04317-2]
46. Wu J, Di W, Wu G, Bei H, Li Z, et al. Scale-out production of extracellular vesicles derived from natural killer cells via mechanical stimulation in a seesaw-motion bioreactor for cancer therapy. *Biofabrication.* 2022;14:45004. [DOI: 10.1088/1758-5090/ac7eeb]
47. Xia B, Gao J, Li S, Huang L, Zhu L, et al. Mechanical stimulation of schwann cells promote peripheral nerve regeneration via extracellular vesicle-mediated transfer of microRNA 23b-3p. *Theranostics.* 2020;10:8974-95. [DOI: 10.7150/thno.44912]
48. Yan L, Wu X. Exosomes produced from 3d cultures of umbilical cord mesenchymal stem cells in a hollow-fiber bioreactor show improved osteochondral regeneration activity. *Cell Biol Toxicol.* 2019;36:165-78. [DOI: 10.1007/s10565-019-09504-5]
49. Min Lim K, Kim S, Yeom J, Choi Y, Lee Y, et al. Advanced 3d dynamic culture system with transforming growth factor- β 3 enhances production of potent extracellular vesicles with modified protein cargoes via upregulation of TGF- β signaling. *J Adv Res.* 2023;47:57-74. [DOI: 10.1016/j.jare.2022.09.005]