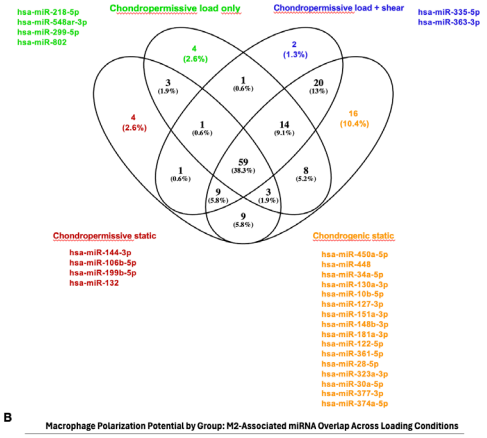
Mechanical loading in bioreactor systems enhances the anti-inflammatory miRNA signatures of MSC extracellular vesicles

Mahammad Gardashli [1,2], Elena Della Bella [3], Manuel Herzog [3], Thomas M Best[1], Martin J Stoddart [3], Dimitrios Kouroupis [1,2]
1 Department of Orthopedics, UHealth Sports Medicine Institute, Miller School of Medicine, University of Miami, Miami, FL, United States
2 Diabetes Research Institute and Cell Transplant Center, Miller School of Medicine, University of Miami, Miami, FL, United States
3 AO Research Institute Davos, Davos, Switzerland
Disclosures: None

INTRODUCTION: Osteoarthritis (OA) is a chronic, progressive joint disorder marked by cartilage degeneration, synovial inflammation, subchondral bone remodeling, and chronic pain, leading to significant personal and societal burden. Affecting over 300 million people worldwide, OA remains a major public health challenge. Current treatments are largely palliative, prompting interest in biologically driven approaches targeting disease mechanisms. Mesenchymal stem/stromal cells (MSCs) are a promising OA therapy due to their immunomodulatory and trophic effects [1,2]. Beyond multilineage differentiation, MSCs secrete anti-inflammatory cytokines (e.g., IL-10, TGF-β), growth factors, and lipid mediators that influence immune cell activity. Extra-synovial MSCs, such as those from the infrapatellar fat pad (IFP), demonstrate enhanced resilience and immunosuppressive activity within the OA joint. MSCs promote macrophage polarization from proinflammatory M1 to reparative M2 phenotypes, a shift critical for reducing synovitis and supporting cartilage repair [3,4,5]. MSC-derived extracellular vesicles (MSC-EVs), recapitulate many therapeutic functions of MSCs, including delivery of regulatory miRNAs, while mitigating risks associated with cell transplantation. Preclinical studies show they preserve cartilage integrity, suppress synovitis, and improve function in OA animal models. Mechanical stimulation is emerging as a key factor in enhancing MSC therapeutic potency. Physiological joint loading promotes anabolic factors secretion and strengthens MSC immunomodulatory activity. Biomechanically relevant priming that has also been shown to boost the anti-inflammatory profile of MSC-EVs[5,6,7]. Our hypothesis is that combining mechanical conditioning with MSC-EV therapy may better mimic the native joint niche, enhancing efficacy and safety in OA treatments.

METHODS: Vertebral bone marrow aspirate (n=5) was collected during spinal surgery with informed patient consent (Inselspital Bern, Switzerland). MSCs were isolated and expanded to passage 3 in α-MEM with MSC-qualified FBS, antibiotics, and bFGF. Macroporous polyurethane scaffolds (⌀ 8 mm, h 4 mm) were pre-soaked in chondropermissive medium and seeded with 5×10^6 MSCs in fibrinogen/thrombin, then placed in PEEK holders. Constructs were assigned to one of four experimental groups: Chondropermissive Static (CP) (chondropermissive medium, no mechanical loading), Chondropermissive Load Only (L) (chondropermissive medium, uniaxial compression: 1 Hz, 10% static + 10% dynamic strain), Chondropermissive Load + Shear (CPLS) (chondropermissive medium, same compression parameters as L plus ± 25° oscillatory shear) and Chondrogenic Static (CH) (chondrogenic medium with TGF-β1, no mechanical loading). Constructs were cultured for 3 days, with mechanical stimulation applied 1 h/day for loaded groups. Conditioned media were collected for EV isolation by ultracentrifugation, RNA extraction, and gene expression profiling (166 miRNA qPCR arrays, GeneCopoeia). Data were normalized to small nucleolar RNA, C/D box 48 (SNORD48), analyzed using $2^{-\Delta\Delta Ct}$, and miRNet interactome mapping. Statistical significance was set at $p<0.05$.

RESULTS: EV miRNA profiling revealed condition-specific signatures. CP had 89 miRNAs (six abundant, cut off >1) linked to immune regulation and macrophage-related transcription factors. The L condition showed 93 miRNAs (seven enriched) targeting immune and myeloid differentiation pathways, with interaction shifts vs. CP. CH displayed the greatest diversity (138 miRNAs, two enriched) with cartilage development and anti-inflammatory associations. CPLS had 107 miRNAs (one enriched) focused on macrophage regulation and cytokine signaling. Comparative mapping (Figure 1) highlighted distinct/overlapping miRNAs comparing loading-dependent differences suggesting mechanical stimulation tunes EV cargos toward immune and cartilage pathways.



Group	IL10 (anti-inflammatory)	CD200R1 (immune suppression)	CD163 (M2 marker)	MRC1 (M2 marker)	NGF (M2 support)	Total M2 miRNA hits
CP	8	8	3	3	7	29
CH	12	9	6	6	9	42
L only	8	6	4	6	7	31
CPLS	10	9	6	6	9	40

Figure 1. (A) The Venn diagram shows distinct and overlapping EVs miRNAs among all conditions, highlighting both unique signatures and a 59 miRNAs core shared across conditions. (B) The table summarizes predicted miRNAs targeting M2 macrophage–associated genes across loading conditions, showing CH with the highest total hits (42), CPLS close behind (40), L-only intermediate (31), and CP lowest (29), indicating mechanical regimens differentially influence EV-mediated immunoregulatory potential.

DISCUSSION: Mechanical loading shaped MSC-EV miRNA immunomodulatory profiles, with the chondrogenic static (CH) condition showing the most robust effects on macrophages signature (42 hits), high IL10, MRC1, CD163, VEGFA, and cartilage markers (WNT, PRG4, TGF-β). Chondropermissive load + shear (CPLS) closely followed (40 hits), combining strong effects on MRC1, CD163, moderate IL10, and cartilage-supportive signaling. These results support that multidimensional mechanical cues, particularly compression plus shear reflecting in vivo joint mechanics, enhance EV cargo toward immunomodulation, and cartilage regeneration.

SIGNIFICANCE: This work demonstrates that specific mechanical regimens shape MSC-EV miRNAs profiles toward targeted immunomodulatory and cartilage-repair pathways. Chondrogenic static and combined load–shear conditions yielded the most favorable predicted profiles. Mechanical priming offers a scalable strategy to enhance EV therapeutic potency for osteoarthritis and other joint disorders.

ACKNOWLEDGEMENTS: Study supported by NIH/NIAMS grant number 5R21AR080388-02 and by AO Trauma.

REFERENCES:
1. Thomas, A.C., et al., J Athl Train, 2017. 52(6): p. 491-496.
2. Kouroupis, D., et al., Stem Cell Res Ther, 2021. 12(1): p. 282.
3. Gardashli, M., et al., Sci Rep, 2024. 14(1): p. 29438.
4. Jones, M., E. Jones, and D. Kouroupis, Bioengineering (Basel), 2024. 11(10).
5. Gardashli, M., et al., Front Bioeng Biotechnol, 2024. 12: p. 1401207.
6. Kouroupis, D. and D. Correa, Front Bioeng Biotechnol, 2021. 9: p. 621748.
7. Drohat, P., et al., Bioengineering (Basel), 2025. 12(5).