Inflammatory Crosstalk With Zone-Specific Meniscus ECM-Bound Nanovesicles Promotes Pathological Remodeling

Sung-Han Jo[1], Se-Hwan Lee[1], and Su Chin Heo[1]
[1]University of Pennsylvania, Philadelphia, PA
josunghan91@gmail.com

DISCLOSURES: S. Jo (N), S. Lee (N), S. Heo (5)

INTRODUCTION: The meniscus is essential for knee function and is a fibrocartilaginous tissue with distinct zonal architecture, providing zone-specialized mechanical and biochemical functions [1]. However, the meniscus possesses limited intrinsic healing capacity, particularly in the avascular inner zone. Injury or degeneration disrupts this finely tuned zonal organization, leading to impaired repair, fibrotic remodeling, and progressive joint degeneration. These pathological changes highlight the need to identify molecular cues that regulate zone-specific meniscus remodeling and fibrogenesis. Recent studies have identified extracelluar matrix (ECM)-bound nanovesicles (NVs) as bioactive carries of proteins, RNAs, and signaling molecules capable of regulating cell behavior and matrix remodeling [2]. Unlike exosome derived from 2D culture, ECM-bound NVs retain tissue-specific molecular signatures and may more accurately reflect the native microenvironment. While NVs are increasingly recognized as mediators of cellular crosstalk and tissue homeostasis, how zone-specific meniscus NVs interact with inflammatory microenvironment, a key driver of meniscal pathology and osteoarthritis, remains poorly understood. Thus, in this study, we investigated the role of zone-specific meniscus ECM-bound NVs (MBVs) in modulating neighboring cell responses under inflammatory conditions, aiming to define their contribution to meniscus degeneration and evaluating their potential as therapeutic targets for zone-specific meniscal regeneration.

METHODS: Meniscus tissues were isolated from juvenile bovines (~3 months old) and cultured in 6-well plates for 3 days under either normal or inflammatory conditions (LPS, 1 μg/mL) **(Fig. 1.A)**. For zone-specific MBVs isolation, inner- and outer-zone tissues were frozen, sectioned (~1 cm), and agitated in hypotonic buffer at 4 °C for 2 days. The homogenized ECM was centrifuged (10,000 g, 10 min, 4 °C), lyophilized (4 days), digested with collagenase, and filtered sequentially (0.45 μm and 0.2 μm). MBV morphology was assessed by scanning electron microscopy (SEM), and particle size distribution was quantified by nanoparticle tracking analysis (NTA). Cellular uptake was visualized by PKH-26 labeling and DAPI counterstaining in macrophages. For functional assays, HUVECs, RAW264.7 macrophages, and bone marrow–derived bovine MSCs were treated with MBVs (5 μg/mL) derived from normal (N) or inflammatory (I) inner and outer zones. Gene expression was assessed by RT-qPCR and statistical significance was determined by one-way ANOVA.

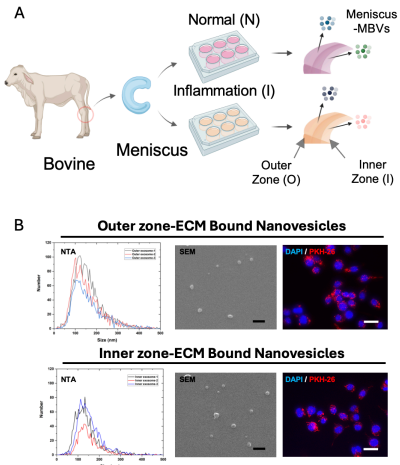


Fig1: (A) Schematic of MBV isolation from juvenile bovine meniscus. (B) Characterization of MBVs by NTA, SEM showing spherical morphology. Scale bar: 200 nm, and PKH-26–labeled uptake in macrophages (red; nuclei in blue) Scale bar: 20 μm.

RESULTS: NTA confirmed that MBVs from both inner and outer zones within the exosomal size range, and SEM demonstrated their round vesicle-like morphology **(Fig. 1B)**. PKH-26 labeling showed efficient uptake by macrophages. MBVs from inner and outer zones exhibited broadly comparable physicochemical features **(Fig. 1B)**. However, interestingly, zone-and condition-dependent differences were found in cellular assays. In HUVECs, only inflammatory inner-zone MBVs significantly upregulated VEGF-A and NOS-3 expression, indicating a pro-angiogenic effect, whereas other MBV groups had no impact **(Fig. 2A)**. In macrophages, inflammatory inner-zone MBVs uniquely increased expression of the inflammatory marker IL-6 and the chemokine MCP-1, promoting immune cell recruitment **(Fig. 2B)** Similarly, MSCs treated with inflammatory inner-zone MBVs showed a marked increase in fibrosis-associated genes (Col1α2 and Col3α1) **(Fig. 2C)**. Collectively, these findings demonstrate that inflammatory inner-zone MBVs exhibit distinct pro-angiogenic, pro-inflammatory, and pro-fibrotic activities. To investigate underlying mechanisms, proteomic profiling was performed. SDS-PAGE revealed compositional differences between outer- and inner-zone MBVs **(Fig. 3A)**. Mass spectrometry identified 555 proteins across groups: 411 in normal outer (NO), 209 in normal inner (NI), 337 in inflammatory outer (IO), and 237 in inflammatory inner (II) MBVs. Notably, 36 unique proteins were found exclusively in inflammatory inner-zone MBVs **(Fig. 3B)**. Gene Ontology enrichment analysis showed that these proteins were associated with extracellular matrix organization, Hippo signaling, intermediate filament organization, and keratinization, suggesting a mechanistic connection to fibrotic remodeling **(Fig. 3C)**.

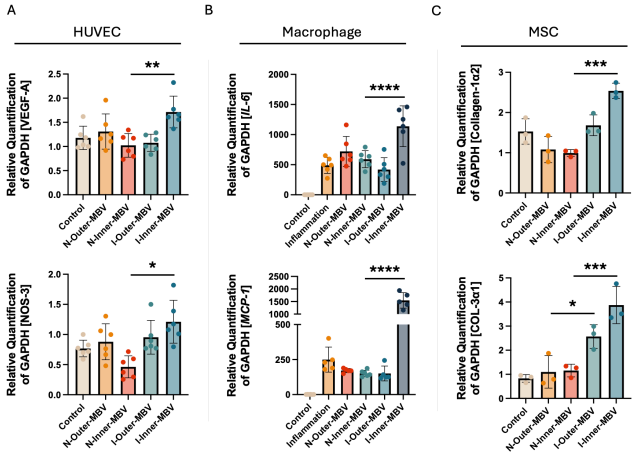


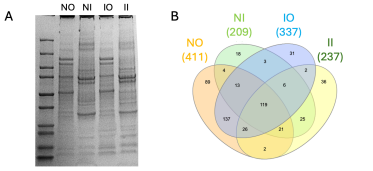
Fig. 2: Functional effects of meniscus MBVs. (A) Angiogenesis-related gene expression (VEGF-A, NOS-3) in HUVECs. (B) Inflammatory and fibrotic genes (IL-6, MCP-1) in RAW264.7 macrophages. (C) Fibrosis-related genes (Col1α2, Col3α1) in MSCs treated with MBVs from different zones/conditions (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

DISCUSSION: This study demonstrates that meniscus MBVs exhibit zone- and condition-specific activities that may contribute to the divergent healing capacities of the inner and outer meniscus. While MBVs from both zones shared comparable morphology and size, only inflammatory inner-zone MBVs drove robust biological responses across multiple cell types, promoting angiogenesis in HUVECs, inflammatory activation in macrophages, and fibrotic gene expression in MSCs. These findings align with the clinical observation that the avascular inner meniscus is particularly prone to poor healing and fibrosis after injury [3]. Proteomic profiling further revealed unique protein cargo enriched in inflammatory inner-zone MBVs, many associated with extracellular matrix remodeling and keratinization, suggesting a mechanistic link to fibrogenesis and maladaptive tissue remodeling. Together, these results highlight MBVs as mediators of zone-specific crosstalk between the meniscus and its inflammatory microenvironment. Together, these results identify MBVs as critical mediators linking the ECM microenvironment to pathological cellular responses, offering new insight into the molecular drivers of meniscal degeneration.

SIGNIFICANCE: This study first identifies inflammatory inner-zone MBVs as drivers of pro-angiogenic, pro-inflammatory, and pro-fibrotic signaling, linking the ECM microenvironment to meniscal degeneration. Targeting MBV-mediated pathways may provide new therapeutic strategies for zone-specific meniscus repair.

REFERENCES: [1] Bilgen, et all. Current Concepts in Meniscus Tissue Engineering and Repair. *AdvancedHealthcare Materials* **2018**, *7*, 1701407. [2] Huleihel, et all. Matrix-Bound Nanovesicles within ECM Bioscaffolds. *Sci. Adv.* **2016**, *2*, e1600502. [3] Hutchinson et all. The Current Role of Biologics for Meniscus Injury and Treatment. *Curr Rev Musculoskelet Med* **2022**, *15*, 456–464,

ACKNOWLEDGEMENTS: This work was supported by the NIH (R01 HL163168) and NSF (CMMI-1548571).



C

GO biological process (BP)	Fold Enrichment	P-value
Establishment of cell polarity involved in ameboidal cell migration	> 100	2.34E-08
Dopamine secretion	> 100	3.32E-05
Hippo signaling	90.28	4.70E-06
Intermediate filament organization	56.04	4.80E-14
Keratinization	47.1	1.56E-06
Regulation of phagocytosis	31.86	7.55E-06

Fig. 3: (A) SDS-PAGE showing distinct protein banding patterns of MBVs from inner and outer zones under normal (N) and inflammatory (I) conditions. **(B)** Venn diagram of proteins identified by mass spectrometry. **(C)** GO enrichment of unique proteins in inflammatory inner-zone MBVs.