Marrow-Isolated Adult Multilineage Inducible Cell-Derived Extracellular Vesicles (MIA-EVs) as Cell-Free Therapeutics for Intervertebral Disc Inflammation

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INTRODUCTION: Intervertebral disc degeneration (IVDD) is a leading cause of low back pain and disability, driven in part by inflammation-induced catabolism in nucleus pulposus (NP) cells[1-3]. Proinflammatory cytokines such as interleukin-1β (IL-1β) disrupt extracellular matrix homeostasis, compromise NP cell survival, and accelerate degenerative changes[4]. Extracellular vesicles (EVs) have emerged as promising cell-free therapeutics capable of delivering bioactive cargo that modulates inflammation and promotes tissue repair in IVDD[5-7]. This study focuses on EVs isolated from a unique subpopulation of adult mesenchymal stem/stromal cells (MSCs) known as Marrow-Isolated Adult Multilineage Inducible cells (MIA-EVs). Our previous work has demonstrated that MIA-EVs carry more potent immunomodulatory and anabolic cargo than conventional MSC-EVs[8]. Here, we evaluate the protective and regenerative potential of MIA-EVs in IL-1β-stimulated NP cells, focusing on their capacity to counteract inflammation and restore a reparative phenotype.

METHODS: Whole bone marrow was obtained from cadaveric donors in accordance with the informed consent guidelines approved by the University of Miami School of Medicine Committee on the Use of Human Subjects in Research. Samples were collected from four donors (female: 12 and 31 years; male: 21 and 60 years). MIAMI cells were cultured under conditions mimicking the bone marrow niche (low oxygen (3% pO_2), low serum, and lipid supplementation) to preserve stemness and enhance function through upregulation of self-renewal transcription factors[9]. For EV collection, upon reaching confluency cells were incubated in EV-depleted medium for 48 h, and conditioned medium (secretome) was harvested, clarified, and subjected to differential ultracentrifugation. To evaluate their functional impact, primary swine NP cells were seeded in 24-well plates and stimulated with 10 ng/mL IL-1β. MIA-EVs were administered at three time points to model distinct therapeutic scenarios: 0 h (protective pre-treatment), 24 h (modulatory co-treatment), and 48 h (regenerative post-treatment). Controls included untreated, EV-only, and IL-1β-only conditions. Secretomes were collected at 24, 48, or 72 h according to treatment schedule, and cytokine levels were quantified using the RayBiotech Quantibody Human Inflammation Array 3, with each analyte measured as the mean of four replicate spots. All conditions were tested in technical duplicates.

RESULTS: MIA-EVs exerted distinct, time-dependent effects on cytokine regulation in IL-1β-stimulated NP cells (Figure 1). IL-1β treatment alone (S3) strongly elevated IL-1β secretion (361 pg/mL), confirming successful inflammation induction. Comparable IL-1β levels persisted in EV pre-treatment (S4, 313 pg/mL) and co-treatment (S5, 336 pg/mL) groups, whereas delayed EV administration (S6) markedly suppressed IL-1β to near-baseline levels (10 pg/mL). Interestingly, TNF RII, an anti-inflammatory decoy receptor, was exclusively detected in S6 (57 pg/mL), suggesting activation of endogenous TNF-regulatory mechanisms specifically triggered by late-phase EV exposure. Anti-inflammatory responses were further supported by IL-1ra, which was suppressed in the IL-1β-only group (S3: 70 pg/mL) but restored to 240 pg/mL and 208 pg/mL in co- (S5) and post-treatment (S6) groups, respectively. Regenerative signaling was reflected in IL-11, which peaked in S6 (4,586 pg/mL), while ECM stabilizing proteins TIMP-1 and TIMP-2 remained elevated in both co- and post-treatment groups, despite partial suppression under inflammatory stress. EV pre-treatment (S4) did not reduce IL-1β or upregulate IL-1ra, but modestly elevated TIMP-1, TIMP-2, and IL-11, suggesting partial priming of NP cells against inflammation. Immune-regulatory IL-2 was suppressed by IL-1β alone but restored above baseline in the co-treatment group (S5), indicating timing-sensitive reactivation of immunoregulatory pathways. Chemokine analysis revealed that MIA-EVs transiently enhanced recruitment signals (e.g., RANTES and MCP-1) during pre- and co-treatment but reduced their levels in S6, consistent with a transition toward inflammation resolution.

DISCUSSION: These results demonstrate that MIA-EVs modulate the inflammatory and regenerative environment of NP cells in a time-dependent manner. EV delivery during the active inflammatory phase (S6) produced the strongest anti-inflammatory and pro-regenerative effects, characterized by near-complete suppression of IL-1β, induction of TNF RII, restoration of IL-1ra, and peak IL-11 levels. This suggests that MIA-EVs are most effective when applied after inflammation has been established, supporting their potential as post-injury therapeutics for IVDD. Co-treatment (S5) provided moderate benefits, restoring IL-1ra and IL-2 while maintaining TIMP expression, indicating early anti-inflammatory and immunoregulatory activity despite persistent IL-1β secretion. Pre-treatment (S4), although ineffective at preventing cytokine induction, modestly enhanced regenerative and matrix-preserving markers, suggesting that prophylactic EV exposure may partially prime NP cells for stress tolerance. Taken together, these findings highlight that timing of EV delivery critically shapes therapeutic outcomes. While early exposure may provide limited priming effects, post-inflammatory intervention with MIA-EVs drives a robust anti-inflammatory shift and regenerative activation. This positions MIA-EVs as promising candidates for cell-free therapies aimed at resolving disc inflammation and preserving NP function in degenerative spinal disorders. Our promising *in vitro* findings foster a next logical step of *in vivo* model testing. Such investigations should address biodistribution, dosing, safety, and efficacy.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates that MIA-EVs exert potent, timing-dependent anti-inflammatory and regenerative effects in IL-1β-stimulated NP cells, with post-inflammatory delivery producing the strongest suppression of IL-1β and activation of reparative cytokines. These findings highlight the translational potential of MIA-EVs as a cell-free therapeutic strategy for IVDD, offering a novel approach to resolve inflammation and preserve disc function in degenerative spinal disorders.

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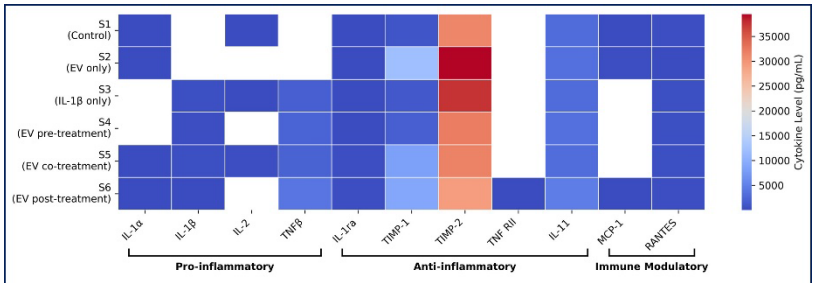

Figure 1. MIA-EVs modulate inflammatory and regenerative cytokine profiles in a time-dependent manner.

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