Synovial Fibroblast Extracellular Vesicles: A Potential Pathway Linking Osteoarthritis to Adverse Cardiac Outcomes

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INTRODUCTION: Growing evidence suggests that cardiovascular disease (CVD) and knee osteoarthritis, two of the most prevalent conditions worldwide, are closely correlated[1,2]. While the two diseases share multiple common risk factors such as age, obesity, family history, smoking, and diabetes mellitus, specific pathophysiological mechanisms between the heart and the knee driving poor disease outcomes remain unclear[1]. Myocardial infarction (MI), which accounts for one-third of CVD cases, is strongly influenced by cardiac fibroblasts (CFs), key regulators of inflammation, repair, and fibrotic remodeling. In parallel, fibroblast-like synoviocytes (FLS) in the knee synovium control immune infiltration and drive the inflammatory environment characteristic of OA. A potential pathway for inter-organ communication between FLS and CFs involves extracellular vesicles (EVs), lipid-bilayer particles that carry stress-dependent cargo and mediate systemic signaling[3]. As osteoarthritis slowly develops over several years, the constant systemic presence of inflammatory EVs derived from the joint may lead to negative outcomes post MI. Given the established clinical link between MI and osteoarthritis, we hypothesize that EVs released by inflamed FLS in osteoarthritis contribute to the inflammatory response of CFs as well as cardiomyocytes **(Figure 1)**.

METHODS: Cell Culture: Healthy human synovial explants were recovered from cadaveric knees (MTF Biologics, Edison, NJ). Synovium tissue was enzymatically digested using collagenase type II and recovered FLS were expanded in α-Minimum Essential Medium (αMEM) supplemented with 10% fetal bovine serum (FBS),1% antibiotic-antimycotic. Cardiac fibroblasts (Promocell) were expanded in Fibroblast Growth Medium-3 with 1% antibiotic-antimycotic. EV Collection: To simulate OA conditions in the joint, FLS were treated with 10 ng/ml IL-1β. Serum free media was added for 24 hours and collected. Media was centrifuged at 2000 g for 20 min, followed by filtration through a 0.22 µm mesh. EVs were isolated using tangential flow filtration (TFF), purified by ultra-centrifugation at 150,000 g and resuspended in 1 mL PBS. Trans-well Co-culture: FLS and CFs were co-cultured in a Trans-well for 3 days, with FLS seeded in the upper chamber and CFs in the lower chamber. Four conditions were tested: (1) FLS + IL-1β, (2) FLS only, (3) α-MEM only and (4) α-MEM + IL-1β. Cells in the trans-well were lysed and RNA was isolated (Qiagen RNeasy Mini Kit). RT-PCR was performed looking at the following genes: Col1A1, Col3A1, TGF-β, α-SMA, IL-6, ADAMST4. EV Treatment: CFs were cultured until confluent, treated with FLS EVs and FLS +IL-1β EVs for 24 hours then RT-PCR on the following genes: α-SMA, IL-6 and IL-1β.

RESULTS: CFs co-cultured with IL-1β–stimulated FLS in a Trans-well system for 3 days exhibited a physiologically accurate inflammatory response **(Figure 2A)**. Gene expression of inflammatory markers IL-6 and ADAMTS4 in CFs were significantly upregulated in response to inflamed FLS while fibrotic markers COL1A1, COL3A1, TGF-β, and α-SMA were downregulated **(Figure 2B)**. Upregulation of these inflammatory markers during the early stage align with acute injury such as myocardial infarction. These results suggest that inflammatory EVs from the knee can contribute to an early inflammatory response of the heart following injury, where pro-inflammatory mediators are upregulated while profibrotic pathways remain transiently downregulated[4,5]. EVs were successfully isolated from both control and IL-1β–treated FLS (IL-EVs). EVs displayed modal sizes of 117.9 nm (IL-1β) and 123.9 nm (no IL-1β), consistent with exosome-sized vesicles **(Figure 3A)**. TEM confirmed their characteristic cup-shaped morphology, while BSA confirmed encapsulation of protein content **(Figure 3B)**. CFs treated with FLS IL-1β EVs showed an increase gene expression of inflammatory markers IL-6 and IL-1β, suggesting that EVs from the inflamed joint are able to influence cardiac inflammation. Interestingly, treatment with FLS EVs also showed an increase in α-SMA gene expression suggesting that there are key differences between EV treatmenet and a Trans-well model. This effect was not observed in CFs treated from normal FLS EVs **(Figure 3C)**.

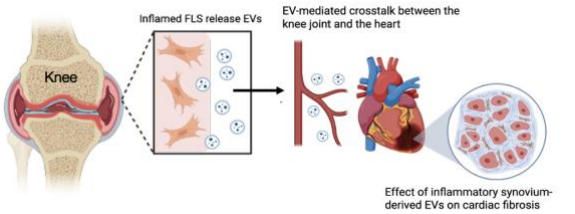

Figure 1. Proposed mechanism of knee and heart crosstalk.

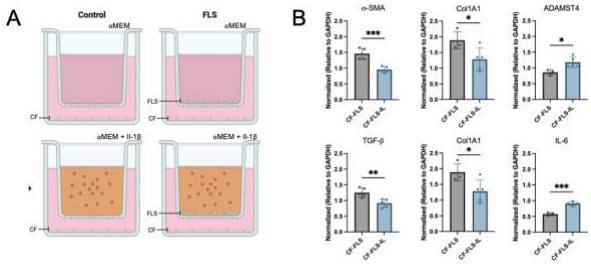

Figure 2: (A) Depiction of Trans-well study with FLS seeded on top +/- IL-1β and CFs on the bottom. (B) After 3 days of co-culture, FLS stimulated with IL-1β downregulate gene expression of key markers for fibrosis in CF. However, key early-stage inflammation markers in MI (IL-6, ADAMST4) are upregulated. Gene expression is normalized to CF only conditions. *p<0.05, **p<0.005, ***p<0.0005.

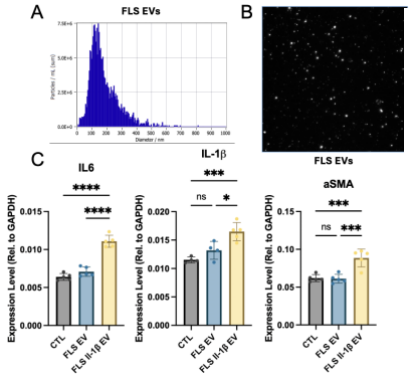

Figure 3. (A) Size distribution of EVs isolated from FLS. (B) TEM images of EVs isolated from FLS. (C) Gene expression in CFs treated with FLS ± IL-1b EVs. *p<0.05 ***p<0.005, ****p<0.00005

DISCUSSION: Evidence shows that synoviocytes release extracellular vesicles that can be found in the synovial fluid and act as indicators for OA severity[6]. Given that the synovium functions as a selective barrier regulating molecular exchange between the joint and the systemic environment, we hypothesize that EVs released by inflamed synoviocytes could contribute to inter-organ communication, particularly influencing the heart. In this study, we first demonstrated that inflamed fibroblast-like synoviocytes (FLS) can induce a physiologically relevant inflammatory response in CFs using a Transwell co-culture system. We next isolated extracellular vesicles (EVs) from both healthy and IL-1β–stimulated FLS and treated CFs with these EV populations. PCR revealed that FLS + IL-1β-derived EVs upregulated inflammatory genes and fibrotic genes in CFs after 24 hours of treatment, an effect not seen in normal FLS EV treatment. Together, these findings support a model in which inflamed FLS during OA can release EVs that propagate inflammatory and fibrotic signaling and potentially contributing to negative CF outcomes. Future work aims to show direct crosstalk between OA and the heart via extracellular vesicles by use of *in vivo* models, the effects of longer-term cultures, and how the presence of cardiomyocytes in a 3D tissue model can affect response.

SIGNIFICANCE/CLINICAL RELEVANCE: As osteoarthritis and cardiovascular disease are two of the most prevalent diseases with a proven clinical association, this study establishes a potential mechanistic link between the heart and the knee joint via cell-cell communication with extracellular vesicles. These findings highlight the concept that osteoarthritis is not only a localized joint disease but may contribute to systemic pro-inflammatory states that can impact cardiovascular outcome allowing us to build an understanding of how various organs in the body crosstalk and interact during disease progression.

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