Synovial Fibroblast Derived Extracellular Vesicles: A Bridge Between Osteoarthritis and Cardiac Disease

Ethan Lee[1], Derek Ning[1], Vanessa YiRan Li[1], Clark Hung[1,2], Ke Cheng[1]
Departments of [1]Biomedical Engineering, [2]Orthopedic Surgery, Columbia University, New York, NY

INTRODUCTION

Knee osteoarthritis and cardiovascular disease (CVD) are among the most prevalent chronic diseases worldwide, and growing evidence suggests they are closely associated [1,2]. Although they share risk factors such as age, obesity, smoking, and diabetes, the specific mechanisms linking cardiac and joint pathology largely remains unclear. Myocardial infarction (MI), which accounts for a third of CVD cases, is crucially modulated by cardiac fibroblasts (CFs) which contribute to the inflammatory and repair response and the formation of fibrotic tissue that can lead to further adverse cardiac remodeling. Similarly, fibroblast-like synoviocytes (FLS) in the knee regulate immune infiltration and joint inflammation in osteoarthritis. A potential cell-cell specific linkage between the heart and the knee involves communication via extracellular vesicles (EVs), lipid bilayer–enclosed carriers of stress-specific particles that mediate cell communication [3]. Given the central role that FLS and CFs play in their respective diseases, the fibroblast-fibroblast crosstalk mediated by EVs between the knee and the heart is particularly interesting. In this study, we hypothesize that EVs released by inflamed FLS in potentially contribute to the inflammatory response of CFs, leading to negative outcomes.

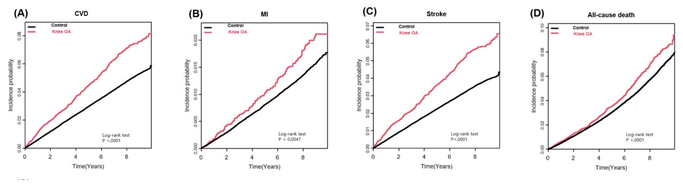


Figure 1: Shared risk factors and disease link between cardiovascular disease (CVD) and osteoarthritis [1]. Populations with knee OA show an increased likelihood of adverse cardiovascular events compared with individuals without OA, highlighting a clinically relevant link between musculoskeletal and cardiovascular disease.

METHODS

FLS Cell Culture: Healthy human synovial explants were recovered from cadaveric knees (MTFBiologics, Edison, NJ). Synovium tissue was enzymatically digested and recovered. FLS were expanded in αMEM + 10% FBS, 1% antibiotic-antimycotic. **CF Cell Culture:** Human cardiac fibroblasts were ordered from Promocell (C-12375) and expanded in Fibroblast Basal Medium 3 + 1% antibiotic-antimycotic. **EV Isolation:** FLS +/- 10 ng/ml IL-1β were treated with serum free media for 24 hours and conditioned media was 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, and then purified by ultra-centrifugation at 150,000 g and resuspended in 1 mL PBS. **Transwell Coculture:** FLS and CF were co-cultured in a transwell for 3 days, with FLS seeded in the upper chamber and CF 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. **RT-PCR:** Cells were lysed and RNA was isolated and circularized (Qiagen RNeasy Mini Kit). RT-PCR was performed on the following genes: Col1A1, Col3A1, TGF-β, α-SMA, IL-6, ADAMST4. **Proliferation Experiment:** CFs were seeded in plates using a stencil to create a central cell-free area and treated with FLS EVs and FLS +IL-1β EVs. Images were captured every 3 hours as cells proliferated. **DNA Picogreen:** CF were treated with FLS EVs and FLS +IL-1β EVs for 24 hours then assayed for DNA content using a dsDNA Kit (ThermoFisher).

RESULTS

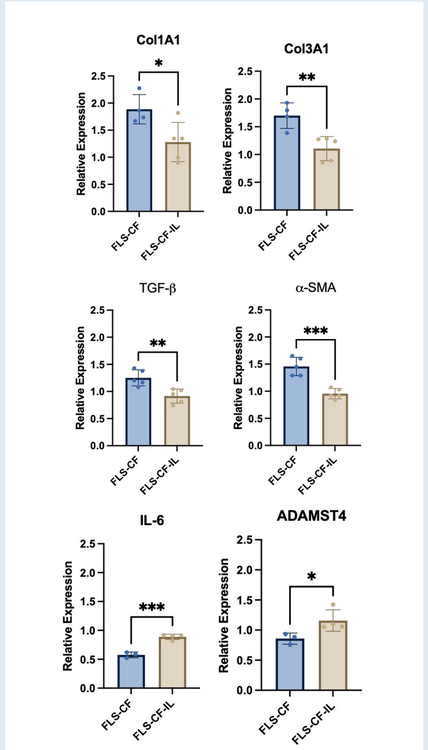
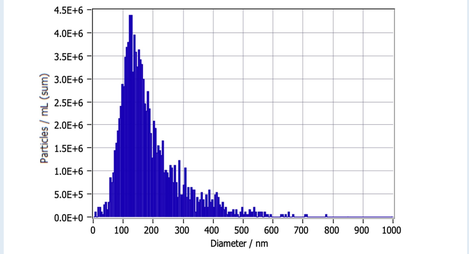
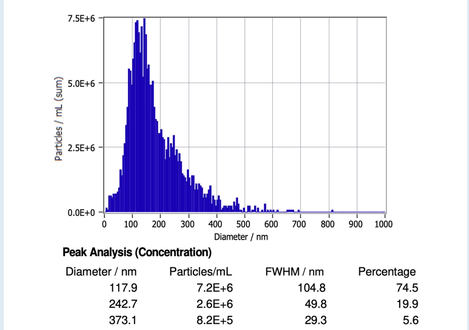


Figure 2: Transwell co-culture of FLS with IL-1β induces changes in CF gene expression. After 3 days of coculture, IL-1β–stimulated FLS (a) downregulate fibrotic markers (Col1A1, Col3A1, TGF-β, αSMA) and (b) upregulate inflammatory mediators (IL-6, ADAMTS4), relative to CFs cultured without FLS. This suggests that activated FLS transmit pro-inflammatory signals to CFs, shifting them towards an inflammatory phenotype over the 3 day time period.



Peak Analysis (Concentration)

Diameter / nm	Particles/mL	FWHM / nm	Percentage
123.9	4.1E+6	102.8	79.8
259.0	1.0E+6	40.7	11.8
412.5	4.7E+5	40.3	5.9
361.1	4.3E+5	12.7	1.6
388.6	4.1E+5	10.0	1.0



Peak Analysis (Concentration)

Diameter / nm	Particles/mL	FWHM / nm	Percentage
117.9	7.2E+6	104.8	74.5
242.7	2.6E+6	49.8	19.9
373.1	8.2E+5	29.3	5.6

Figure 3: Presence of EVs confirmed by particle size analysis. EVs were successfully isolated from control fibroblast-like synoviocytes (FLS; top) and FLS treated with IL-1β (IL-EVs; bottom). Presence of EVs was confirmed by particle size analysis, showing diameters around 100 nm (123.9 nm, 79.8%; 117.9 nm, 74.5%).

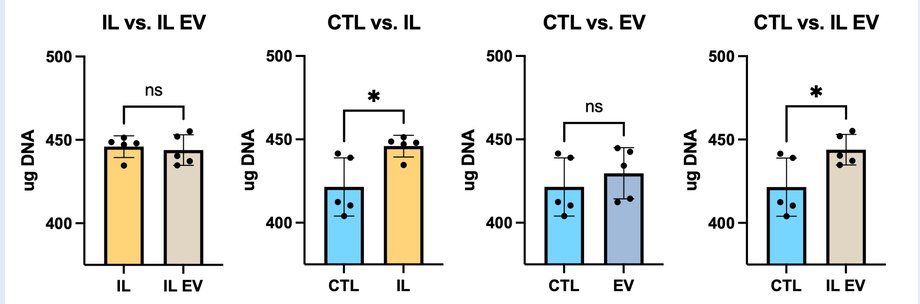
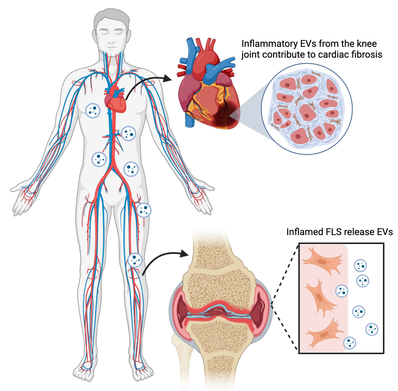


Figure 4: CF proliferation DNA assay. Treatment with IL-1β and IL-1β–stimulated extracellular vesicles (IL-EVs) resulted in a significant increase in CF proliferation, whereas EVs isolated from control fibroblast-like synoviocytes (FLS-EVs) had no significant effect. These results suggest that EVs may serve as carriers of proliferative signals induced by IL-1β.

CONCLUSIONS

In summary, the study demonstrates that inflammatory FLS are able to alter the response of CFs through both direct co-culture and EV signaling. As indicated by Figure 2, in a transwell FLS-CF co-culture, relative to αMEM only controls, inflammatory markers (IL-6, ADAMST4) in cardiac fibroblasts were more strongly upregulated in conditions with IL-1β. Fibrotic markers (Col1A1, Col3A1, TGF- β, α-SMA,) were downregulated in response to IL-1β + FLS treatment (Figure 2). These results mirror the early inflammatory phase of CFs, involving increased inflammation and decreased fibrosis suggesting that the presence of inflamed FLS may lead to more significant negative outcomes in CFs. EVs were then successfully isolated from control FLS and FLS treated with IL-1β (IL-EVs) (Figure 3). Presence of EVs were confirmed by diameter of particles being around 100 nm. (123.9 nm, 79.8%, 117.9 nm, 74.5%). In a proliferation assays, IL-1β treatment and IL-EVs resulted in an increase in CF proliferation, a key outcome of cardiac fibrosis. Control FLS EVs did not effect cardiac fibroblast proliferation significantly (Figure 4). Together, these findings support a model where IL-1β stimulates FLS to release EVs that propagate inflammatory and fibrotic signaling, potentially contributing to negative CF outcomes. Longer-term cultures and additional functional assays will be important to confirm whether these EV-mediated effects lead to full fibrotic transition and lead to negative myocardial outcomes.



ACKNOWLEDGEMENTS

I would like to sincerely thank my mentors, Derek Ning and Vanessa Li, for their guidance, support, and invaluable feedback throughout this project. I am also deeply grateful to Professor Clark Hung and Professor Ke Cheng for providing the resources and collaborative environment that made this research possible. I would also like to thank the Undergraduate Research Fellowship (URF) program for the opportunity to present this research.

References: [1] https://pubmed.ncbi.nlm.nih.gov/26464295/ [2] https://www.nature.com/articles/s41598-023-29581-1#Sec12 ; [3] Cheng+, Drug Discov, 2022.