Developing a T Cell and Synovium Co-Culture System to Assess the Effect of Adaptative Immunity on the Pathogenesis of Osteoarthritis

Ratna Sharma[1], Neeraj Sakhrani[1], Anna-Liisa Sepp[1], Derek Ning[1], Arvind Nandakumar[1], Claire Pinnie[1], Roshan P. Shah[2], Lance C. Kam[1], Clark T. Hung[1,2]
[1]Department of Biomedical Engineering, [2]Department of Orthopedic Surgery, Columbia University, New York, NY; rs4241@columbia.edu

Disclosures: RS (N), NS (N), AS (N), DN (N), AN (N), CP (N), RPS (N), LCK (N), CTH (1; MTF. 8; Deputy Editor of JOR, Editor for Orthopedic Research and Reviews)

INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease characterized by synovial inflammation and cartilage damage.[1] The synovial pro-inflammatory environment is mediated by fibroblast-like synoviocytes (FLS) and resident macrophage-like synoviocytes through cytokine, degradative enzyme, and immune cell-recruiting chemokine production.[2,3] Synovium is highly vascularized, producing lubricating molecules such as hyaluronan (HA), with abnormal accumulation of HA found in arthritic joints.[4-6] During inflammation, HA interacts with immune cells, cytokines, and cell surface receptors to modulate immune responses.[7,8] T lymphocyte cells (CD4+) are present in areas enriched with FLS, composing over 20% of the immune cells that infiltrate the synovial membranes of OA patients.[8,9] However, mechanisms underlying T cells-FLS interactions in OA pathogenesis remain poorly understood. We hypothesized that by co-culturing T cells with FLS using direct contact and indirect transwell systems, we could model immunologic crosstalk of the synovial microenvironment, gaining novel insights into OA pathogenesis and joint inflammation under diseased conditions. To understand cytotoxic effects of T cells, Study 1 characterized FLS viability in co-culture with T cells. Study 2 assessed the release of nitric oxide (NO) and HA into media following exposure to T cells. In Study 3, the expression of proinflammatory cytokines, matrix degradation, and surface T cell receptors was assessed.

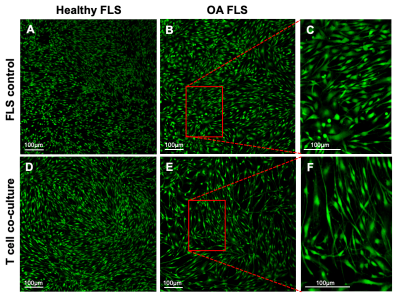

Figure 1: Cell viability staining (live: green and dead:red) of (A-C) FLS alone and (D-F) FLS cultured with T cells.

METHODS: Cell Isolation: Healthy human synovium was obtained from MTF Biologics (N=3; only male cadaver samples were used due to donor availability) and OA explants were harvested during total knee arthroplasty (N=1; only female tissue was received due to donor availability). Explants were digested to isolate FLS. Healthy human T cells were obtained from NYBC and isolated via RosetteSep Human T cell enrichment and Ficoll-Paque (N=2; one female and one male donor). T cells were used within 6 days after aCD3/aCD28 activation. Direct Contact Co-Culture: FLS were expanded using αMEM supplemented with 10% FBS, 1% antibiotic/antimycotic (AA), and 5 ng/mL FGF-2. Following overnight attachment, T cells were cultured onto the FLS in 50% v/v RPMI 1640 supplemented with 10% heat-inactivated FBS and αMEM supplemented with 10% heat-inactivated FBS and 1% AA. Transwell Co-Culture: T cells and FLS were cultured on 0.3 μm pore polyester membrane inserts. FLS were expanded on the plate bottom using αMEM supplemented with 10% FBS, 1% AA, and 5 ng/mL FGF-2. Following overnight attachment, basal media was replaced with αMEM supplemented with 10% heat-inactivated FBS, 1% AA, and 5 ng/mL FGF-2. T cells were seeded on the apical side of the insert using RPMI 1640 supplemented with 10% heat-inactivated FBS. Study 1: Following direct contact culture for 48h, live/dead staining of the FLS-T cell system was performed to assess cell viability using Calcein AM and Ethidium Homodimer. Study 2: Media samples from the co-culture systems were assayed for NO and HA release. Study 3: RNA was isolated from the co-cultures. Gene expression was analyzed using RT-qPCR for markers of proinflammatory cytokines (IL1β, IL-6), matrix degradation (MMP3, MMP1), and surface T cell receptors (CD3E, CD3G). Statistics: Target genes were normalized to GAPDH. Comparisons of transwell and direct contact systems as well as healthy and OA FLS were made using two-way ANOVA with Tukey HSD post-hoc tests (α=0.05).

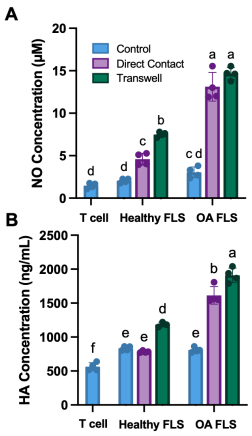

Figure 2: Media analysis of (A) nitric oxide and (B) hyaluronan in co-culture. Groups not sharing a letter are significantly different from each other (p<0.05).

RESULTS: Cell viability remained unchanged with T cell co-culture in both healthy and OA FLS (**Fig 1A,D** and **1B,E** respectively**)**. Visual inspection reveals morphological elongation of the FLS cells when cultured with T cells (**Fig 1C, 1F).** NO was elevated in healthy FLS co-cultured directly and indirectly with T cells compared to FLS controls (p=0.0090 and p<0.0001 respectively; **Fig 2A**). OA FLS in direct contact with T cells and cultured in a transwell system significantly increased NO release (p<0.0001; **Fig 2A**) compared to OA FLS controls. OA FLS cultured with T cells displayed a greater increase in NO release compared to healthy FLS cultured with T cells (p<0.0001 for direct contact and transwell system; **Fig 2A**). HA release was elevated in the healthy co-culture transwell system compared to healthy FLS controls (p=0.0001; **Fig 2B**). OA FLS cultured directly and indirectly with T cells significantly increased HA release (p<0.0001; **Fig 2B**) compared to OA FLS controls. Compared to healthy FLS, OA FLS co-cultured in both systems displayed significantly greater release of HA (p<0.0001; **Fig 2B**). Baseline HA release was lower in T cell controls compared to healthy and OA FLS controls (p= 0.0015 and p= 0.0044 respectively; **Fig 2B**). Gene expression showed significant differences across co-culture systems and cell type. Proinflammatory cytokines, IL1β and IL-6, increased with T cell co-culture (direct contact and transwell), with a greater increase in OA FLS than healthy FLS (**Fig 3A**). Matrix degradative marker expression increased, with MMP3 significantly upregulated in healthy and OA FLS co-cultured with T cells compared to controls (**Fig 3B**). Elevation of T cell receptor gene expression was observed in T cell only controls (**Fig 3C**).

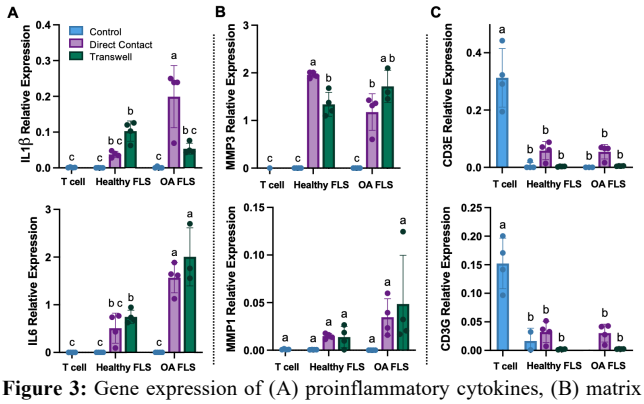

Figure 3: Gene expression of (A) proinflammatory cytokines, (B) matrix degradation enzymes, and (C) T cell surface receptor proteins. Groups not sharing a letter are significantly different from each other (p<0.05).

DISCUSSION: Healthy and OA FLS exposed to T cell co-culture, appeared elongated, suggesting these immune cells alter FLS growth but not viability. Media analysis demonstrated that T cell co-culture increased inflammatory activity, characteristic of OA development.[10] Gene expression of FLS showed T cells promote inflammation and ECM degradation, reflecting features of OA.[11] Modulation of cell-cell interactions via direct contact and transwell co-culture systems reveal T cells alter FLS towards an OA phenotype.

SIGNIFICANCE: By co-culturing T cells and FLS, we attempt to incorporate adaptive immunity into an *in vitro* model of the synovium to explore the role of T lymphocytes in OA. This model reveals T cells increase the inflammatory activity of FLS, characteristic of the OA disease state.

REFERENCES: 1. Hamada+ *Arthritis Rheum* 2016. 2. Culemann+ *Nature* 2019. 3. Smith *Open Rheumatol J* 2011; 4. Hui+ *Wiley Interdiscip Rev Syst Biol Med* 2012. 5. Wells+ *Arthritis Rheum* 1992. 6. Band+ *Osteoarthritis Cartilage* 2015. 7. Ruppert+ *Immunol Res* 2014. 8. Jiang+ *Physiol Rev* 2011. 8. Kang+ *Matrix Biol Plus* 2022. 9. Pessler+ *Clin Rheumatol* 2008. 10. Sakhrani+ *Front Bioeng Biotechnol* 2022. 11. Li+ *Exp Mol Med.* 2021.

ACKNOWLEDGEMENTS: NSF GRFP, MTF Biologics