Roles of Multi-Tissue Crosstalk in Engineered Healing of Avascular Meniscus Tears under Inflammation

Hun Jin Jeong, Solaiman Tarafder, Bozhi Chen, Elen Zhu, Chang H. Lee
Columbia University, New York, NY Email: hj2607@cumc.columbia.edu

Disclosures: Hun Jin Jeong (N), Solaiman Tarafder (N), Bozhi Chen (N), Elen Zhu (N), Chang H. Lee (N)

INTRODUCTION: The knee joint is a complex multi-tissue organ comprising the meniscus, articular cartilage, subchondral bones, synovial membrane, ligaments, and infrapatellar fat pad (IFP). These components in the synovial joint play important roles in the maintenance of joint homeostasis, disease initiation, and progress through multi-tissue crosstalk. The synovial membrane consists of intimal and subintimal layer, which not only produces the hyaluronan and the lubricin essential for maintaining the proper joint function but also secretes pro-inflammation cytokines in polarized microphages such as IL-1β and TNF-α as reaction of the inflamed by joint tissue injuries. Similarly, the IFP plays roles in the initiation and progress of joint diseases through inter-tissue communications mediated by several adipokines such as leptin, adiponectin, leptin, and resistin. Although these components in the synovial joint are pivotal for the metabolism of joint tissues, crosstalk between joint tissue has been rarely investigated, and only a few studies have been reported as focused on investigating osteoarthritis (OA). To understand the roles of multi-tissue crosstalk in meniscus healing, we have established a 3D knee joint model consisting of a joint chamber containing meniscus explant, the 3D bioprinted synovial membrane (syM), and tissue-engineered fat pad (eAT), separated by semi-permeable membranes allowing transportation of secreted cytokines. Our previous study confirmed the functionality of our 3D joint model by confirming the viability and bioactivities of the syM and eAT and their signaling interactions under inflammatory stimulation. This study was designed to investigate the effect of co-culture of syM and/or eAT on the avascular meniscus healing induced by our well-established bioactive glue. Our central hypothesis is that crosstalk between synovium, fat pad, and meniscus plays important roles in meniscus healing associated with inflammatory modulation.

METHODS: _3D bioprinted synovial membrane (syM):_ Macrophages were derived from THP-1 human monocytes, and synovial mesenchymal stem/progenitor cells (syMSCs) were isolated from surgically removed synovium of anonymous adult donors (20 to 25 years old) as per our previous methods. Then two layers of gelatin methacryloyl (GelMA) bioinks (12% w/v, 0.4% photo-initiator) were printed using a lab-made extrusion type 3D bioprinter (macrophage layer and syMSCs layer; 1×10^6 cells/ml for each cell type). The two-layered membrane was bioprinted in a 15 ×15 mm rectangular shape with a thickness of 0.6 mm. The bioprinted constructs were then cross-linked using UV (365nm) light for 30s. Polarization of macrophages in the 3D bioprinted syM was confirmed by applying lipopolysaccharide (LPS, 100 ng/mL) + human interferon-γ (IFN-γ, 100 ng/mL) (M1) or recombinant human interleukin-4 (IL-4, 40 ng/mL) + recombinant human IL-13 (20 ng/mL) (M2), followed by confocal microscopy for M1 and M2 marker expressions. _Engineered adipose tissue (eAT):_ Human adipose tissue-derived stem/progenitor cells (ATCC®, Manassas, VA) were embedded (8×10^6 cells/ml) in the 3D printed polycaprolactone (PCL) scaffold ($10 \times 7 \times 5$ mm^3) with 3% collagen bioink (Advanced Biomatrix, Carlsbad, CA), followed by 4 wks culture in adipogenic differentiation media. Adipose tissue formation was confirmed by Oil-Red O (ORO) staining. _Meniscus explant:_ As per our prior methods, bovine meniscus explants of 1/3 inner zone with full-thickness longitudinal incisions were prepared, and fibrin gel cross-linked with genipin (FibGen) with 100 ng/ml CTGF and 10 mg/ml of TGFβ3 encapsulated in PLGA µS was applied to the defect site with syMSCs suspended in media. After 1 wk of culture, the meniscus explants were transferred to the 3D joint model. _In vitro joint model:_ Polydimethylsiloxane (PDMS) mold was fabricated using a 3D printed mold with curing at 60°C for 6 hours. The semi-permeable membranes were then installed to compartmentalize the chambers for different joint tissues, and syM and eAT were placed in each tissue compartment. Single tissue groups, syM with meniscus (syM/M), meniscus with eAT (M/eAT), and all three tissues (syM/M/eAT) were prepared. To simulate inflammation, 10 ng/ml IL-1β was applied in the joint chamber during meniscus healing. After 4 wks, meniscus healing was evaluated by our established methods, cytokine levels were measured using multiplex cytokine assays, and gene expressions were measured by qRT-PCR. After 4 wks of co-culture, the phenotype maintenance of each tissue type was evaluated by histology (e.g., ORO) and immunofluorescence with M1 and M2 markers.

RESULTS: After 4 wks of co-culture in the 3D joint model (**Fig. 1A**), no sign of structural degradation was observed for all the three joint tissues (**Fig. 1B-D**). syM showed M1 and M2 polarization upon respective treatment after 4 wks multi-tissue co-culture (**Fig. 1B**) and eAT exhibited abundant lipid droplets (**Fig. 1C**). In addition, multi-tissue co-culture in the joint model showed no interference in the healing of avascular meniscus tears in the explant treated by bioactive glue (**Fig. 1D**). When stimulated by IL-1β, meniscus healing was notably disrupted in meniscus alone and syM/M groups (**Fig. 2A**), likely associated with inflammation-induced matrix degradation. Interestingly, co-culture with eAT (M/eAT) and all tissues (syM/M/eAT) showed improved meniscus healing even under IL-1β stimulation (**Fig. 2A**). Adiponectin (ADIPOQ) and leptin expressions were significantly reduced by IL-1β in M/eAT co-culture, but such reduction was significantly attenuated in all three tissue co-cultures (**Fig. 2B**). Similarly, PPARG-γ expression was significantly reduced by IL-1β in M/eAT group of which effect was significantly attenuated in syM/M/eAT co-cultures (**Fig. 2B**). IL-1β and IL-6 expressions in syM were significantly increased when treated by IL-1β in syM/M groups, whereas the level of pro-inflammatory cytokines were reduced when meniscus were co-cultured with syM and eAT (syM/M/eAT). Consistently, the total concentrations of IL-1β in the joint chamber over time (>5 days after the initial IL-1β application) were significantly reduced in syM/M/eAT samples.

DISCUSSION: The present findings suggest that the reduced inflammatory responses in co-culture with eAT may attenuate the detrimental effect of IL-1β on meniscus healing in the 3D joint model. The anti-inflammatory function of eAT toward syM may be associated with its adipokine expression. Interestingly, we observed the adipokine expressions in eAT were not only affected by IL-1β but also co-culture with syM, suggesting bi-directional interaction between those tissues. Although additional investigations are warranted to better understand the signaling connecting three joint tissues, this study confirms the functionality and effectiveness of our 3D joint model to study multi-tissue crosstalk in meniscus healing. The limitations of this study include the potential tissue degradation in a long-term co-culture, especially when stimulated by IL-1β, and the un-described effects of CTGF and TGFβ3 released from bioactive glue on syM and eAT metabolism and signaling.

SIGNIFICANCE: Our 3D _in vitro_ joint model has significant potential to serve as an efficient experimental platform to delineate the complex roles of interactions between joint tissues in meniscus injury and healing. The identified roles of fat pads in meniscus healing associated with synovium may have significant implications in development of bioengineering strategies for regenerative healing of knee meniscus.
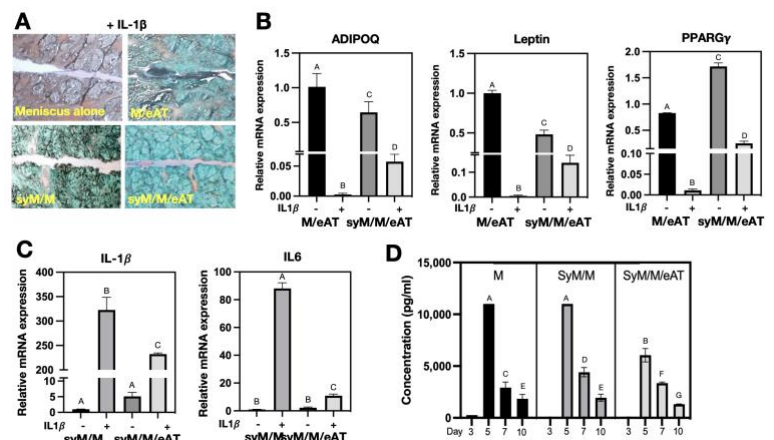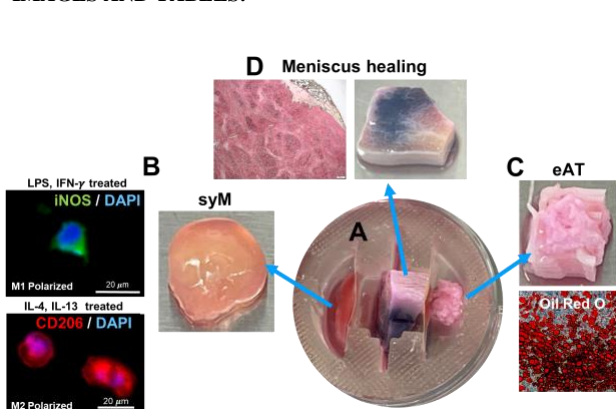
IMAGES AND TABLES:

Fig. 1. Phenotype maintenance of syM, meniscus, and eAT for up to 4 wks co-culture. All tissues show no sign of structural degeneration (**A**). Macrophages in the bioprinted Syn_Mem harvested after 8 wks co-culture showed proper polarization in response to M1 and M2 stimulation for 24 hours (**B**). eAT showed abundant lipid droplets after 8 wks co-culture (**C**). Meniscus explant also showed a healing of defect (**D**).

Fig. 2. Meniscus healing with bioactive glue was interfered by IL-1β but multi-tissue co-culture attenuate such effect (**A**). Multi-tissue co-culture affects the expression pattern of adipokines PPARGγ in eAT depending on IL-β stimulation (**B**). Similarly, IL-1β and IL6 expressions in syM were affected by IL-1β stimulation and multi-tissue co-culture (**C**). Multi-tissue co-culture significantly reduced the total concentration of IL-1β in the joint chambers in a timely manner (**D**). n = 5 per group; different letters indicate significant difference p<0.01.