Adipocyte-Mediated OA Inflammation in a Joint-on-Chip Model

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INTRODUCTION: Osteoarthritis (OA) is the most common joint disease worldwide and a leading cause of disability in older adults; OA affects 32.5 million adults in the U.S. and results in pain, functional limitations and financial loss [1]. Despite advances in treatment, there are no FDA-approved disease-modifying drugs that prevent, slow, or reverse OA progression, and current management is limited to pain relief and preserving joint function [2]. Obesity is a significant risk factor, conferring up to a 4.6-fold higher risk of OA [3]. While once attributed mainly to mechanical loading, OA also develops in non-weight-bearing joints, implicating systemic factors. Accordingly, the endocrine activity of adipose tissue has gained attention, as adipocytes release adipokines and inflammatory cytokines that influence joint physiology and OA pathogenesis [4]. In this study, we use human MSCs (hMSCs) from OA patients to investigate the influence of adipose tissue on OA pathology. We hypothesize that the presence of adipose tissue modulates the lineage commitment of hMSCs, particularly affecting their chondrogenic and osteogenic potential via paracrine signaling and cellular crosstalk.

METHODS: Different components of the joint-on-chip tissue, including adipose tissue (AT) and synovial membrane (SM), were generated by differentiating hMSCs and co-culturing them within a single bioreactor designed for organ-on-chip applications. Differentiated hMSCs were encapsulated in 15% methacrylated gelatin (GelMA) at 500,000 cells/scaffold for 3D culture to form an AT and SM tissue. In this system, adipocytes simulate the adipose tissue, while fibroblasts mixed with HUVECs mimic the synovial membrane. This engineered microenvironment was co-cultured with MSCs to assess chondrogenesis, and with human blood–derived macrophages to evaluate osteogenic potential. To determine the influence of adipocyte inflammation on the joint microenvironment, IL-1β (10 ng/mL) was applied to the joint-on-chip system for 21 days to model OA. Assessment of these tissues was accomplished using histology, RT-qPCR, and Luminex assay. All experiments were performed in triplicate, with data presented as mean ± standard deviation.

RESULTS: In the RT-qPCR analysis, the presence of adipose tissue, synovial ECM markers *FN1* and *COL3A1* significantly decreased, and inflammation enhanced the decline, which suggests adipose-derived signals may compromise synovial integrity ($p < 0.01$). Conversely, angiogenic markers (*FGF2*, *VEGF*) were upregulated in the presence of AT and further increased with inflammation, suggesting a pro-angiogenic microenvironment. Chondrogenic markers such as *COL10A1* increased in groups containing AT; *COMP* decreased dramatically with and without adipocytes, reflecting a trend towards cartilage degradation. Osteogenic markers *SPP1* increased in an inflammatory environment, with *SPARC* decreasing; adipogenic PNIN and PPARG also decreased in inflamed adipocytes. Luminex assay results showed that *adiponectin* was elevated early (day 2). Furthermore, there was a significant increase in *MCP-1* in the shared medium from the joint-on-chip system treated with IL-1β ($p < 0.01$).

DISCUSSION: Our findings highlight the pivotal role of endocrine activity of adipose tissue in the regulation of joint microenvironment, both in healthy and inflamed tissues simulating a miniature knee joint. The joint-on-chip incorporates bone, cartilage, fibrous, and adipose tissues, and exhibits physiologically relevant pathological changes when the entire joint microenvironment experiences IL-1β induced inflammation. Also, this study evaluated how adipose tissue modulates the lineage commitment of MSCs, particularly affecting their chondrogenic and osteogenic potential via paracrine signaling and cellular crosstalk. Through investigating the mechanisms by which adipocyte-derived factors drive synovial inflammation and influence joint integrity, we can elucidate the critical crosstalk between adipose tissue and joint compartments.

SIGNIFICANCE/CLINICAL RELEVANCE: This organ-on-chip model may serve as a physiologically relevant *in vitro* platform to study joint regeneration and disease mechanisms in OA.

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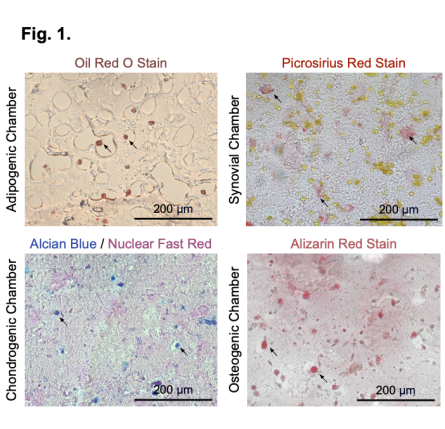

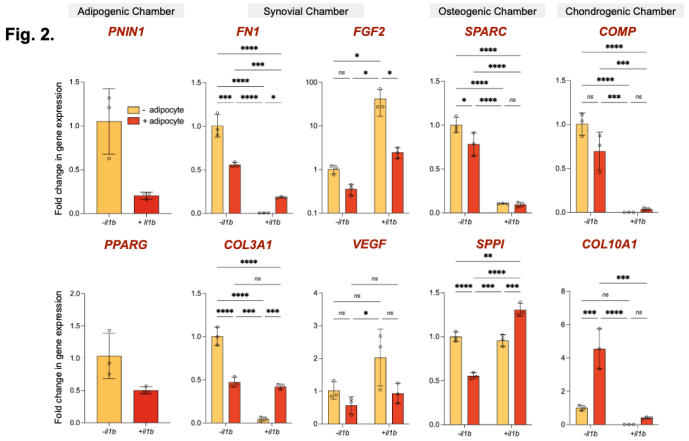

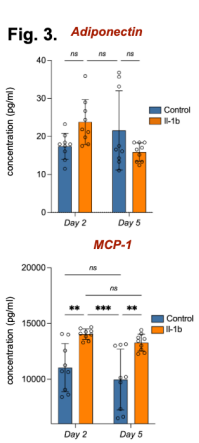

Figure 1. Histological staining confirming specific markers in the components of the joint-on-chip. **Figure 2.** Expression of key marker genes in all four tissue components. **Figure 3.** The concentrations of selected adipokines in the shared medium collected from control and inflamed joint-on-chips.