Human iPSC-based microphysiological system for studying adipose-cartilage crosstalk in obesity-associated osteoarthritis

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INTRODUCTION: Obesity is a growing global epidemic that has profound implications for multiple organ systems, including the musculoskeletal system. Obesity is a well-established risk factor for chronic conditions such as cardiovascular disease, type 2 diabetes, and also osteoarthritis (OA). Aside from excessive mechanical load on the knee joint due to increased body mass, there is little known about the mechanisms underlying the association between obesity and OA. However, it is thought that obese adipose tissue contributes to OA via the production of pro-inflammatory cytokines and adipokines, causing a chronic low-grade state of inflammation. Understanding these interconnected metabolic, inflammatory, and biomechanical effects is essential for developing targeted interventions to mitigate OA progression in individuals with obesity. Leveraging the unique strength of microphysiological systems (MPSs), the **objective** of this study is to engineer a fat-cartilage MPS incorporating induced pluripotent stem cell (iPSC) derived obese-like adipose tissue and iPSC derived cartilage to study the biochemical effects of obese-like changes of adipose tissue on cartilage.

METHODS: *Engineering Obese-like Adipose Tissues*: Human iPSCs were differentiated into induced multipotent progenitor cells (iMPCs) and then differentiated into adipocytes using a two-step process. At 80% confluency, iMPCs were treated with an adipogenic medium for three days in a monolayer culture. On the third day, cells were detached and encapsulated in 15% methacrylated gelatin (GelMA) and cultured for 21 days in an MPS system perfused with a second adipogenic medium at a flow rate of 2 µL/min. At the end of the differentiation period, to promote adipocyte hypertrophy commonly seen in obese adipose tissue, the engineered adipose tissue was supplemented with sodium palmitate (NaPA), a free fatty acid, for 14 days. Assessment of these tissues was accomplished using Oil Red O staining, qRT-PCR, and Bulk RNA Sequencing. *Engineering Cartilage Tissues:* iMPCs were encapsulated in 15% gelMA and underwent differentiation for 28 days in a chondrogenic media containing bone morphogenic protein -4 (BMP-4) for the first 7 days of culture, and transforming growth factor-β3 (TGF-β3) for the full 28 days. Media was perfused through the tissues in an MPS at a flow rate of 2µL/min. Tissues were collected for histology, immunohistochemistry, and qRT-PCR. *Biochemical effects of obese-like changes of adipose tissue on cartilage:* After 14 days of treatment with NaPA, the adipogenic MPS was connected to the completely differentiated cartilage MPS (**Fig. 2A**). Soluble factors from the adipose tissue were perfused through the cartilage MPS via a shared media flow for 14 days. Afterward, tissues were collected for histology, immunohistochemistry, and qRT-PCR. Statistical assessments were conducted using a confidence interval of 95% and a p-value of 0.05. A sample of size of three has been used for the current work, as this data is still in preliminary stages. The iPSCs used in the work were differentiated into iMPCs and sourced from a male donor.

RESULTS SECTION: After 38 days in culture, the engineered obese-like adipose tissue had increased lipid droplet size compared to the control, as quantified by lipid droplet diameter (**Fig. 1A &B**). Additionally, qRT-PCR demonstrated an increase in adipokines such as *adiponectin* (*ADIPOQ*), *adipsin*, *lipoprotein lipase* (*LPL*), and *Peroxisome Proliferator-Activated Receptor Gamma 2* (*PPARG-2)* (**Fig. 1C**). Furthermore, there was a significant increase in the expression levels of inflammatory cytokines, such as *Interleukin (IL)- 6 (IL-6)*, *Tumor necrosis factor- α (TNF-a)*, and *IL-1β*, in the obese-like tissue (**Fig. 1D**). Bulk RNA Sequencing identified 994 upregulated and 875 downregulated genes, including key adipokines and inflammatory cytokines such as *Stearoyl-CoA Desaturase* (*SCD*), *IL-6*, *IL-8/CXCL8*, *Vascular Cell Adhesion Molecule 1* (*VCAM1*), in the obese adipose tissues (**Fig. 1E**). KEGG and GO enrichment pathway analysis indicated adipokine and NF-kB signaling pathway activation (**Fig. 1F**). In the cartilage tissue, the perfusion of adipokines and cytokines secreted from obese-like tissues resulted in the upregulation of inflammatory cytokine genes, such as *IL-8, IL-1β,* and *TNF-α* and an increase in matrix degrading enzymes, including *Matrix Metalloproteinase (MMP)-1* and *A Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS)-4* and *5* (**Fig. 2B**). Ongoing studies are using histology and immunohistochemistry to further examine the changes in cartilage.

DISCUSSION: The results indicate that NaPA successfully induced an inflammatory, obese-like state while preserving key adipose features. In addition, the increased production of inflammatory cytokines in the obese-like adipose tissue did affect the cartilage constructs after 14 days of shared media flow. Herein, we demonstrate the development of an MPS platform that can be used to study obesity-associated OA and test potential therapeutics. We are currently assessing the potential therapeutic effects of several disease-modifying drugs, such as Metformin, on our fat-cartilage MPS.

SIGNIFICANCE/CLINICAL RELEVANCE: Obesity associated OA is not well understood and complex. Through investigating the mechanisms behind the role of obesity associated adipokines and inflammatory cytokines and subsequent cartilage inflammation/degradation, we can begin to understand the crosstalk of obese-like adipose tissue and the cartilage, providing a novel platform to study obesity-associated OA in humans, define druggable targets, and screen new therapeutics. The use of patient-derived cells will also enable the development of personalized treatments.

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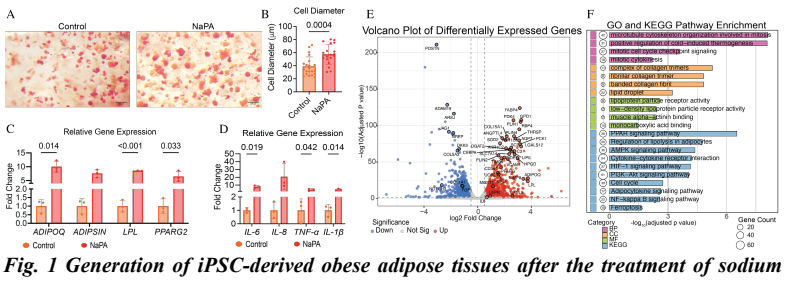

Fig. 1 Generation of iPSC-derived obese adipose tissues after the treatment of sodium palmitate (NaPA). *(A) Oil Red O Staining for lipid droplet formation in iPSC-derived fat treated by NaPA or vehicle control. Scale Bar: 100µm. (B) Quantification of lipid droplet diameter in control and NaPA groups. (C) qRT-PCR assessing expression levels of representative adipogenic genes. N=3. (E) qRT-PCR assessing expression levels of selected pro-inflammatory genes in control vs NaPA treated groups. N=3. (E) Volcano plot demonstrating 994 upregulated and 875 downregulated genes in control vs NaPA treated groups. (F) GO and KEGG Pathway analysis of top pathways associated with differentially expressed genes upregulated in the NaPA treated group.*

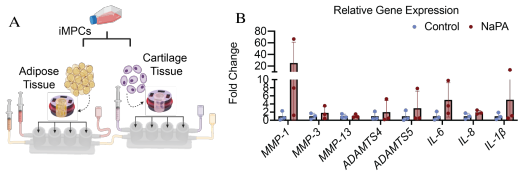

Fig. 2 Detrimental impact of secretome from obese adipose tissues on cartilage. *(A) Schematic detailing the connection of the adipose tissues to the cartilage via a shared bottom medium flow to make the fat-cartilage microphysiological system. Of note, flow direction can be switched so we can analyze the interaction between fat and cartilage under different contexts. Shared medium can also be used to examine the changes of biomarkers. (B) qRT-PCR assessing expression levels of MMP-1, 3, &13, ADAMTS4&5, and IL-1β, 6&8. in cartilage perfused with condition medium from the control adipose (Control) or NaPA treated (NaPA) adipose tissues. N=3.*