Macrophage modulates inflammation, angiogenesis, and fibrosis in synovial membrane.

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INTRODUCTION: Synovium lines the inner surface of the joint capsule and produces lubricated and viscous fluid in articular joints. The synovium responds quickly to injury and plays an important role in immunity. For instance, wear debris from orthopaedic materials such as ultra-high molecular weight polyethylene (PE) activates various cells and secretes inflammatory factors which cause chronic inflammation including osteoarthritis. Chronic inflammation is centrally driven by macrophages, whereas the pro-inflammatory (M1) phenotype induces inflammatory responses and the anti-inflammatory (M2) phenotype responds to acute inflammation resolves. Besides, extensive angiogenesis occurs in the sub-lining, and inflammatory cells derived from the bone marrow migrate to the joint from the sub-lining and through the lining layer barrier. Here, we investigated the correlation between inflammation and angiogenesis in the vascularized synovial membrane using a microphysiological system with the hypothesis that macrophage phenotype regulated inflammation, angiogenesis, and fibrosis. Gene profiles, self-assembled vascular networks, and inflammatory responses were investigated.

METHODS: Human bone marrow-derived MSCs were maintained in a fibrogenic medium (Advanced DMEM supplemented with 5% FBS, 1% GlutaMAX, 1% antibiotic-antimycotic, and 50 µg/mL Ascorbic acid) for four weeks to obtain fibroblasts. GFP-positive human umbilical vein endothelial cells (HUVECs) were obtained from Angio-Proteomie. Primary macrophages were obtained from deidentified healthy donors. 20 ng/mL IFNγ and 10 ng/mL LPS were applied to obtain the M1 phenotype, while 20 ng/mL IL-4 was used to obtain the M2 phenotype. Cells were seeded in a fibrin-photocrosslinkable methacrylated gelatin (GelMA) hybrid scaffold. Fibroblasts and HUVECs were either coculture with M1 macrophages (M1 coculture group) or M2 macrophages (M2 coculture group). In addition, contaminated PE particles (cPE) were added to mimic chronic inflammation. Vessel networks were acquired by the Leica confocal microscope. A Human Cytokine Array/Chemokine Array 48-Plex (Eve Technologies, Calgary, Canada) was used to determine the level of cytokine secretion. RNA sequencing was conducted to compare transcriptional profiles.

RESULTS: Comparison of the gene expression pattern between the M1 coculture group and the M2 coculture group provides clues to understanding the cellular mechanism of how the immune system regulates synovium. There were 642 genes (differentially expressed genes (DEGs)) that showed a significant change on day 7. As shown in Figure 1 (A-E), gene ontology (GO) enrichment analysis revealed that genes related to ossification, regulation of cellular response to growth factor stimulus, and response to BMP were significantly enriched in the M1 coculture group, while genes related to neutrophil activation and neutrophil mediated immunity were the most enriched in the M2 coculture group. We also monitored the neovascularization process by confocal microscope and the results are summarized in Figure1 (F-G). Vessel length and diameter were increased as the incubation time increased. At week 1, short thin tubes were distributed sporadically in the scaffold. Then, the tubes elongated and branched out, and a hierarchical branching structure was present at week 3 and 4. Vessels in the M2 coculture group revealed a more robust structure. Statistical analysis shows there were longer and thicker vessels in the M2 group than that in the M1 group. Angiogenesis and inflammation are independent biological processes. The culture medium was collected on day 7, and the cytokine comparison is shown in Figure 4. A higher secretion of MIG/CXCL9 and IP-10 was observed in the M1 coculture group, which indicates a pro-inflammatory environment. On the contrary, elevated levels of IL-1RA, IL-18, IL-10, and MCP-3 were detected in the M2 group.

cPEs were added to the system, and 1956 DEGs were detected between the M1 coculture group and the M2 coculture group. In the presence of cPE, extracellular matrix organization, extracellular structure organization, and ameboidal-type cell migration were highly enriched in the M1 coculture group, and neutrophil activation and leukocyte proliferation were highly enriched in the M2 coculture group. Meanwhile, vessel volume in the M2 coculture group was significantly higher than that in the M1 coculture group.

DISCUSSION: We focus on coculturing fibroblasts, polarized macrophages, and endothelial cells in a microphysiological 3D system to examine the relationship between chronic inflammation and angiogenesis. Macrophage-derived cytokines play a key role in chronic inflammation responses and angiogenesis in pathological situations. Although inflammation promotes angiogenesis in several ways, we found that anti-inflammatory phenotypes of macrophages accelerate angiogenesis. Anti-inflammatory treatments hold potential protective effects and could serve as a therapeutic modality.

SIGNIFICANCE/CLINICAL RELEVANCE: Patients develop chronic inflammation and fibrosis of the synovial membrane in osteoarthritis (OA) and post-total joint arthroplasty. Comprehensive analyses of vascular formation, cytokine secretion, and gene expression suggest putative biological pathways for immune modulation to mitigate chronic inflammation and subsequent synovitis.

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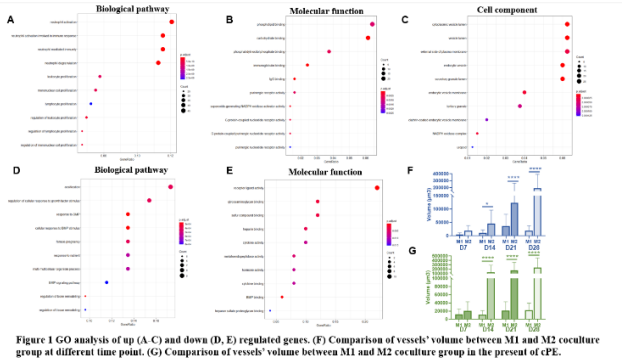

Figure 1 GO analysis of up (A-C) and down (D, E) regulated genes. (F) Comparison of vessels' volume between M1 and M2 coculture group at different time point. (G) Comparison of vessels' volume between M1 and M2 coculture group in the present of cPE.

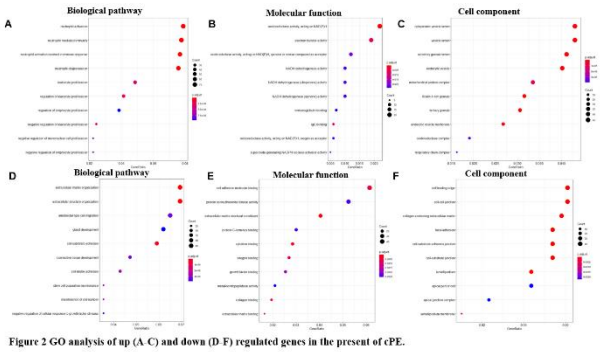

Figure 2 GO analysis of up (A-C) and down (D-F) regulated genes in the present of cPE.

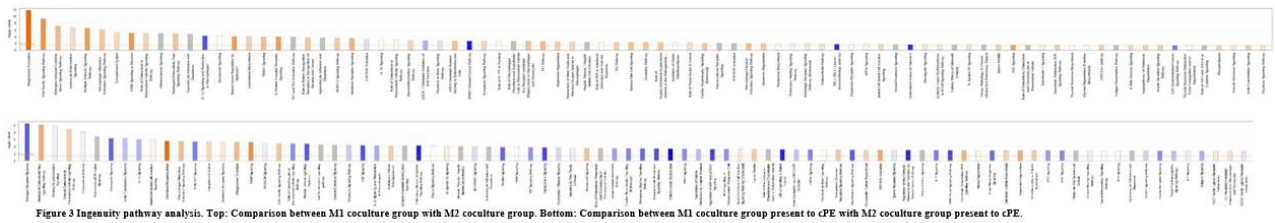

Figure 3 Ingenuity pathway analysis. Top: Comparison between M1 coculture group with M2 coculture group. Bottom: Comparison between M1 coculture group present to cPE with M2 coculture group present to cPE.

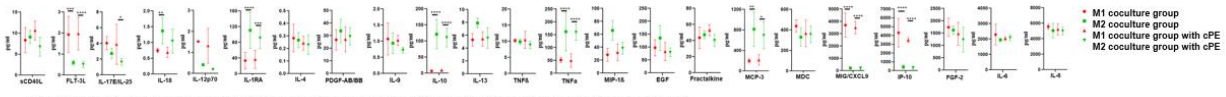

Figure 4 Cytokine comparison among M1 and M2 coculture groups with or without cPE treatment. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$