Identifying the Impact of Osteoarthritis Synoviocytes and Cellular Senescence on Stem Cell Differentiation Potential and Altered Cellular Functions of Neighboring MSCs in 3D Human Joint Tissue Platform

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Statement of Purpose: Cellular senescence is an irreversible cell cycle arrest that the major role is to suppress tumor formation and to promote wound healing.[1–3] Cellular senescence also contributes to age-related cellular phenotypes which cause onset of several human diseases. Osteoarthritis (OA) is characterized by accumulated senescent cells in cartilage in response to cellular aging or acute injury. Cellular interactions between senescent cells and neighboring cells within the local and distant joint tissue have to be further explored. This study investigates the mechanistic role human fibroblast-like synoviocyte (HFLS) and the accumulation of senescent cells in altering OA cellular microenvironment of the 3D printed tri-culture bioreactor (TCB) system. The TCB creates organotypic complex co-culture platform that spatially controls the crosstalk of neighboring cell types within the recreated joint tissue layers in the hydrogel construct generated with Gelatin Methacrylate (GelMA).

Methods: qRT-PCR analysis was conducted for detecting MMP transcriptional markers in normal and OA HFLS. Protein analysis by western blot identified the protein expression levels of MMP marker in the whole cell lysates of normal and OA HFLS. Intrinsic replicative senescence was induced *in vitro* by subculturing MSCs to passage 9. The pH sensitive β-galactosidase staining was done in early (P2) and late (P9) passage MSCs using X-gal substrate and incubate at 37°C (without CO_2). Co-culture of HFLS and hMSCs (osteo-chondral progenitors) in transwell insert in tissue culture plates recreated a cellular microenvironment of human joint tissue. Immunofluorescence assay in MSCs co-cultured with normal or OA HFLS for detected human cartilage marker, hAggrecan. Also DNA damage marker, pH2AX, was detected by immunofluorescence signals. 3D printed bioreactor design provides a dynamic culture which provides a controlled exchange of nutrients. Hydrogel scaffold consists of three distinct cell layers embedding HFLS, chondrogenic-differentiating hMSCs and osteogenic-differentiating hMSCs in 7% GelMA. This cell encapsulated scaffold was inserted to tri-culture bioreactor (TCB) which provides a unidirectional flow of the culture media to maintain and induce the differentiation.

Results: OA HFLS indicated an upregulation of MMP gene and protein expressions (Figure 1A&B). To mimic OA joint tissue cell microenvironment, the cellular senescence was induced in hMSCs which reflects the shortening of telomere and indicated by senescence associated (SA)- β-galactosidase staining in the passage 9 of hMSCs. In addition, reduced level of chondrogenesis was observed in late passage MSCs that were co-cultured with normal or OA HFLS in the transwell system (Figure 1D). Another defective cellular function in chondrogenesis-induced late passage hMSC co-cultured with OA HFLS in the transwell system was characterized by the DNA damage molecular marker such as phosphorylated H2AX, also referred as γH2AX (Figure 1E). Synovium-chondrogenic-osteogenic tissue-mimicking layers of GelMA construct in the TCB (Figure 1F) allowed the gradual diffusion of molecular factors to the neighboring cell types in different layers. In summary, altered stem cell function is associated with the presence of senescent chondro-progenitors and OA HFLS.

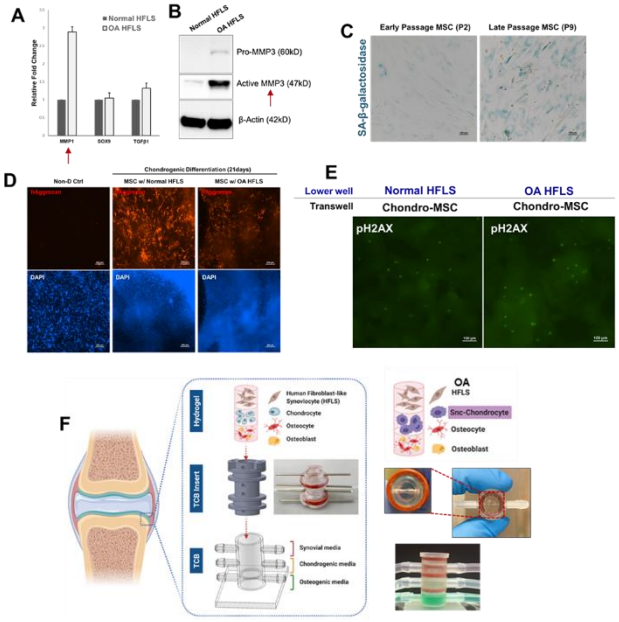

Figure 1. The presence of senescent chondro-progenitors and cellular crosstalk with OA HFLS affects stem cell differentiation capacity of neighboring hMSCs. A. Relative fold change of mRNA expressions in MMP9, SOX9 and TGFβ. **B.** Western blot analysis for the detection of pro- & active MMP3 and β-actin as a loading control. **C.** Senescence-associated β-galactosidase staining in early (P2) and late (P9) passage hMSCs. **D.** Human Aggrecan (Red) fluorescent signals in Chondrogenic differentiated MSCs that are co-cultured with normal or OA HFLS in the transwell for 21 days. DAPI is a counterstain indicating nucleus. **E.** DNA damage marker, pH2AX, immunofluorescence signals (Green) in chondrogenic differentiated MSCs co-cultured with normal and OA HFLS in transwell. **F.** Engineered 3D human joint tissue cellular microenvironment which promotes the cellular crosstalk between synovium and osteo-chondral layers within the GelMA insert scaffold is placed inside the Tri-culture bioreactor (TCB) which creates three distinct chambers for different types of culture media.

References:

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