Assessing Human Articular Cartilage and Osteoarthritis Transcriptome Layer-by-Layer

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Disclosures: -

INTRODUCTION: The Centers for Disease Control and Prevention (2024) have stated that over 32.5 million adults in the US are affected by Osteoarthritis (OA), which deteriorates human articular cartilage, causing pain and limiting activity. Attempts to engineer tissue to replicate the anisotropic tri-layered multi-functional have been limited at best nor is there much success in creating the scaffolded microenvironment mimicry required to accomplish this goal. To achieve the desired multi-functional tissue an understanding of the genes that fully characterize this healthy stratum in needed as a first step in designing tissue culture engineering protocols to reproduce the biomechanical and biochemical striated tissue that's needed for implantation. Not only is the genetic transcriptome not fully characterized to form regenerative normal human articular cartilage (NHAC) engineered tissue, but it is equally not known for diseased tissue to contrast genetic conditions we want to avoid. The primary objective in this study is to generate the spatial transcriptomic signature of NHAC and OA cartilage (OAC) in a typical cross-sectional tri-layered construct. We are applying bulk RNA sequencing to compare gene expression profiles of mesenchymal stromal cells (MSCs) and the multifunctional NHAC and OA tissues.

METHODS: NHAC samples were collected from femoral condyle bones of 3 donors, ages 13, 25, and 27. OAC samples were collected from tissues extracted during total knee arthroplasty from 3 patients, ages 64, 74, and 76. The samples were formalin-fixed and paraffin-embedded (FFPE) and stained with Safranin O/Fast green and by immunofluorescence with antibodies for SOX9, CD44, RUNX2, ACAN, COL2A1, and lubricin protein targets. We are using NanoString technology to generate bulk RNA sequencing results from our samples. The data are being analyzed using Seurat and Python-based RNA sequencing packages to generate gene expression profiles tied to the state of cartilage health of tissue donors and to do so spatially covering the full cartilage section across surface, middle, and deep zones.

RESULTS SECTION: While immunofluorescence staining of NHAC samples shows anticipated expressions of chondrogenic proteins, including SOX9, CD44, ACAN, COL2A1, and Lubricin, these are expressed at lower levels in the staining of OAC samples than in the NHAC samples. Of more importance for our group is the spatial mRNA transcriptome for these proteins. Our group seeks to regenerate the anisotropic organization of tri-layered NHAC from MSCs through a specialized bioreactor with a gradation in shear stress exposure, oscillating hydrostatic pressure and TGF-β growth factor. We are comparing cartilage generated in our reactor to NHAC results.

DISCUSSION: Conventional staining of cartilage shows a tri-layered NHAC structure while OA structure deviates from that. We expect to present bulk RNA sequencing to reveal genetic transcriptomics that correlate with variations in chondrogenic proteins and physical properties in NHAC and OAC in superficial, middle, and deep zones. We will present results for MSC differentiation in our specialized bioreactor with the goal of generating multifunctional hyaline cartilage constructs with gene expression profiles that correlate more closely to those of the tri-layered transcriptome in NHAC. OAC transcriptome results are expected to reveal new information about the layer-by-layer impacts of degenerative conditions. These findings are expected to contribute significantly to advancing regenerative strategies for cartilage repair.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides transcriptomic insights into normal and osteoarthritic human cartilage and evaluates MSC-derived constructs, which is clinically relevant for developing targeted regenerative therapies for osteoarthritis.

REFERENCES: -

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