Single-Cell RNA Sequencing of the Male and Female Human Discoid Meniscus Following Partial Meniscal Resection

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INTRODUCTION: Discoid meniscus is an abnormal variant of the human knee meniscus that presents with increased coverage of the tibial plateau, higher levels of vascularization, and abnormal matrix organization. Additionally, case reports have indicated that the discoid meniscus may be able to re-grow following surgical resection[1-4]. Tears in the meniscus are the most common intra-articular knee injury and their proper repair can help prevent early onset osteoarthritis. Single cell RNA sequencing (scRNA-Seq) is a powerful tool to understand the gene expression of cell populations, which has only begun to be utilized to better understand the heterogeneity of the human knee meniscus[5,6]. Additionally, only a single study[5] has conducted scRNA-Seq of the discoid meniscus and used osteoarthritic menisci from only female donors greater than 50 years of age. Herein we present scRNA-Seq analyses of discoid and non-discoid resected tissue from male and female donors aged 11-18 prior to degenerative changes. To investigate the impact of processing parameters on identified genes and populations, we utilized two common analysis workflows Seurat[7-11] and Partek[12]. *The objective of this study was to perform single cell RNA sequencing on discoid and non-discoid menisci to elucidate the functional biology of discoid meniscus and potentially a capacity for avascular repair*.

METHODS: Meniscus tissue was acquired through an IRB from Children's Mercy Hospital (Kansas City, MO) (IRB Exemption #STUDY00000746). Non-discoid menisci were acquired during partial meniscectomy surgeries, and discoid samples were obtained following saucerization. Tissue was digested in Liberase™ for 3 hours followed by centrifugation and cell harvest. Male (n=2 non-discoid, n=3 discoid) and female (n=2 non-discoid, n=2 discoid) meniscal cells were thawed and encapsulated using a 10X Chromium controller and sequenced using an Illumina NovaSeq at a read depth of 50,000 reads per cell. Seurat and Partek were used in parallel to compare the impact of parameters and process. Analysis parameters are listed in **Figure 1 A-B.** Uniform Manifold Approximation (UMAP) embeddings were used to visualize data for both workflows. All reported genes are significantly different with a false discovery rate (FDR) of < 0.001 in Partek and an adjusted P value of <.001 in Seurat.

RESULTS SECTION: ***Seurat vs Partek*:** Seurat analysis of meniscus samples yielded 9 different clusters and Partek was then given 9 clusters as the starting parameter for k-means **Figure 1 A-B.** Clustering of fibrochondrocyte subpopulations (Figure 1 A Clusters 0-4,6,8 and Figure 1 B clusters 1-6) supports prior literature indicating several closely related matrix producing fibrochondrocyte populations and a small but distinct cluster of proliferating cells[5,6]. Partek and Seurat analysis both illustrated similar clusters for an immune cell population, however only Seurat's clustering method was able to successfully isolate the proliferating fibrochondrocyte subpopulation (Cluster 7, **Figure 1A**). **Figure 1E** demonstrates the proliferating cell population previously described[6] was successfully re-capitulated by Seurat analysis with key marker genes STMN1 and CDK1. ***Discoid vs Non-Discoid:*** **Figure 1A** Cluster 3, **Figure 1B** Cluster 4, and **Figure 1D** demonstrate key extracellular matrix genes that exist primarily in Seurat cluster 3 and Partek cluster 4 and are upregulated in discoid such as *CILP*, *CHAD*, *COMP*, and *ASPN*. Interestingly, *CILP* expression was consistent across female samples but highly upregulated in male discoid. Discoid samples also demonstrated several genes related to matrix catabolism namely chitinase 3-like-1 (*CHI3L1*) and matrix metalloproteinase (*MMP3*). Our work isolated a novel subgroup of immune cells, separated from the rest of the cell populations by CD74 and HLA-DRA expression seen in Figure 1F.

DISCUSSION: Our results illustrate the heterogeneity of fibrochondrocyte populations in the human inner knee meniscus, which has traditionally been assumed as more chondrocyte-like cells. Our analysis additionally re-capitulated and added to the understanding of cell populations in the human knee meniscus, with a small proliferative cell population marked by STMN1[6]. However, our analysis is the first scRNA-Seq study to suggest a subpopulation of the meniscus with a distinct immune phenotype. Several key matrix genes that are understood as markers of osteoarthritis (OA) but are not functionally annotated in the meniscus or other cartilage-like tissues are suggested for further study here as they are upregulated in discoid and may be responsible for matrix disorganization including *CILP*, *COMP*, *CHAD*, *ANGPTL7*, *DCN*, and *LUM*.

SIGNIFICANCE/CLINICAL RELEVANCE: These results provide an improved understanding of genes involved in matrix disorganization in the discoid meniscus and provide new targets for tissue engineering. The identification of an immune cell population also presents an opportunity to target and separate the cells for parallel investigation of the immune component and its impact on overall repair of the human knee meniscus.

REFERENCES:[1] T. Soejima, *OPEN ACCESS*, p. 4, 2013.[2] J. Baek, et al. *Clin. Case Rep.*, vol. 10, no. 3, Mar. 2022 [3] S.-B. Han et al, *Knee Surg. Sports Traumatol. Arthrosc.*, vol. 26, no. 8, pp. 2278–2281, Aug. 2018[4] M. I. Stein et al, *Am J Orthop* 2013 p. 4. [5] H. Sun *et al., J. Cell. Physiol.*, vol. 237, no. 5, pp. 2469–2477, May 2022 [6] W. Fu *et al., eLife*, vol. 11, p. e79585, Dec. 2022 [7] Hao et al. Nature Biotechnology (2023) [8] Hao and Hao et al. Cell (2021) [9] Stuart and Butler et al. Cell (2019) [10] Butler et al.Nat Biotechnol (2018) [11] Satija and Farrell et al. Nat Biotechnol (2015) [12] Partek Inc. (2020). Partek® Flow® (Version 10.0) [Computer software]

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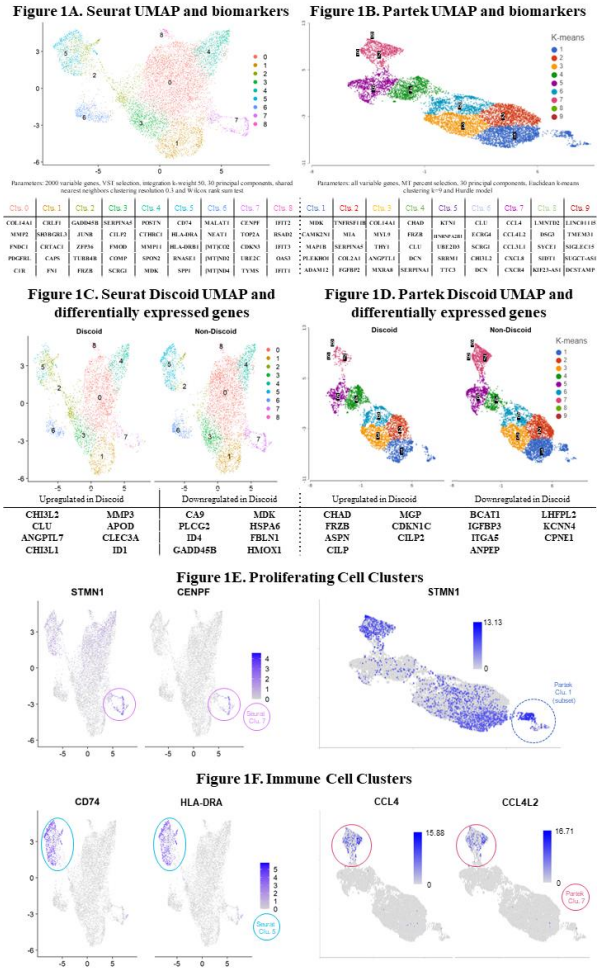

Figure 1 A-B. Seurat and Partek UMAPs of total cell population (discoid and non-discoid) and top 5 biomarkers for each cluster. **Figure 1 C-D.** Seurat and Partek UMAPs split by discoid and non-discoid samples and top 7 differentially expressed genes. **Figure 1 E.** proliferating cell cluster. **Figure 1 F.** Immune cell clusters