Application of spatial transcriptomics to human osteochondral explants and intervertebral disc tissue to enable identification of molecular pathways during disease.

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INTRODUCTION: Spatial transcriptomics holds potential to unravel the complex pathophysiology of diseases including osteoarthritis, and intervertebral disc degeneration. However, to date its application to these tissues has been limited to mouse and early human embryonic tissues. Application in the non-foetal human has been hampered by low metabolic activity of cells resulting in low transcriptional reads. The presence of calcified tissues necessitates the application of decalcification procedures which can further decrease the readable RNA content. Finally, these tissues are notoriously difficult to retain slide attachment due to high proteoglycan content and tissue swelling during the procedures. These limitations have hampered the application of this technique to human cartilage, bone and intervertebral disc samples. Here, we describe an optimised and validated methodology to apply spatial transcriptomics to human bone, cartilage, and intervertebral disc samples.

METHODS: Osteochondral samples were collected from patients undergoing knee replacement, osteolytic bone samples from those undergoing revision hip surgery, and intervertebral disc samples from those undergoing discectomy for nerve root compression. Tissue samples were fixed in 10% neutral buffered formalin, and where bone was present decalcified in 20% EDTA in DEPC treated water until clear by µCT imaging prior to embedding in paraffin wax using DEPC treated solutions. To confirm RNA integrity and quantity, RNA was isolated from 20µm sections using Qiagen RNA extraction kit for paraffin sections, and DV200 determined using an Agilent bioanalyzer. Tissue samples were selected based on DV200 and RNA quantity for spatial transcriptomic analysis. Adhesion of sections for spatial transcriptomics was optimised testing slide types, drying method and downstream transcriptomics protocol. Spatial transcriptomics was performed using 10x genomics Cytassit Visium v2 platform, and libraries sequenced by Novogene. The analysis pipeline was optimised using Spaceranger, Loupe Browser, and Seurat in R, using a brain sample in parallel as a validated tissue.

RESULTS: The optimal methodology involved mounting 7µm paraffin sections using DEPC treated water onto Suprafrost Plus slides, drying at 37◦C for 48hrs and then in a silica gel container at room temperature for 2 weeks. Spatial transcriptomics methodology was adjusted to decrease agitation during staining steps and careful application of all solutions to retain tissue adhesion. Analysis pipelines required manual tissue alignment using the 10x Loupe browser and manual spot selection to avoid folded or lifted tissue regions. Whist mean reads per spot within osteochondral, osteolytic membrane and disc samples were lower than brain tissues (Osteochondral samples: ~25; Osteolytic membrane: ~1000, Disc: ~150; Brain: 2,500), the total number of genes identified were similar (17,500-18,000 genes in all tissue types). The method was able to resolve genes that were spatially differentially expressed across all tissues, identifying between 3 and 7 differential cell clusters dependant on tissue type, with key differential genes identified between clusters (Figure 1). Spatially expressed genes were identified across common cell clusters such as spatially expressed matrix genes (Figure 2).

DISCUSSION: To the authors knowledge, this is the first report of the successful adaptation of 10x spatial transcriptomics to adult human bone, cartilage, and intervertebral disc, which are low transcriptome and high extracellular matrix rich tissues. Analysis is ongoing to investigate differential expression across pathophysiological status, including paracrine activity between anatomically adjacent versus the more distant clusters, to give insights into the functionally relevant networks and pathways involved.

SIGNIFICANCE AND CLINICAL IMPORTANCE: The application of spatial transcriptomics to mature human bone, cartilage and disc will enable deeper understanding of disease pathogenesis and provide avenues to much needed clinical translation.

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Figure 1: Example heat maps showing differentially expressed genes between clusters within spatial transcriptomics of A: Osteochondral explant; B: Osteolytic bone; and C: Herniated intervertebral disc tissue.

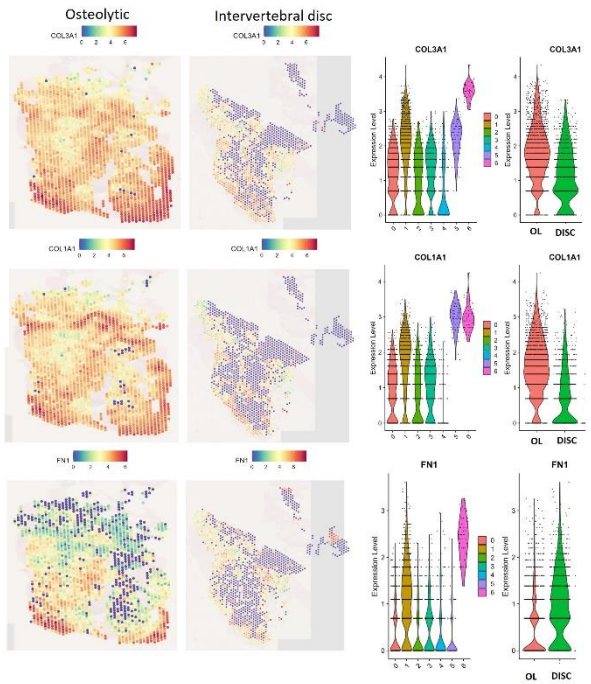

Figure 2: Spatially expressed genes across common cell clusters, examples shown for osteolytic and disc tissues. Example spatial gene expression shown for COL3A1, COL1A1 and Fibronectin