Establishment of an *ex vivo* osteochondral explant and synovium co-culture system

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INTRODUCTION: In joint-related diseases such as osteoarthritis (OA), the interplay among cartilage, subchondral bone, and synovial tissue plays a crucial role in both the onset and progression of the disease. Therefore, it is necessary to consider the effect of bone and synovium when studying cartilage repair and regeneration. *Ex vivo* joint models have emerged as indispensable tools to investigate the complex interactions within the joint environment, providing a platform for modeling cartilage-related disease and drug-testing. Significant variations exist among studies utilizing *ex vivo* joint models regarding the diverse levels of glucose employed in the tissue culture medium. This study aimed to investigate the effect of different levels of glucose in the establishment of an inflammatory *ex vivo* joint model including cartilage, subchondral bone and synovium tissues.

METHODS: Bovine osteochondral explants and synovium (8 mm in diameter) were collected from the stifle joint. *Ex vivo* co-cultures were conducted in two different culture media: DMEM high glucose (HG, 4.5 g/L), and DMEM low glucose (LG, 1g/L). An inflammatory condition was simulated using IL-1β and TNF (1 ng/mL). At day 7 of culture, release of glycosaminoglycan (GAG) and nitric oxide (NO) were measured using Dimethylmethylene Blue (DMMB) and Griess assay, respectively. Expression of mRNA transcripts encoding anabolic (*ACAN*, *COL1*, *COL2*, *VEGF*), catabolic (*MMP13*, *ADAMTS4*, and *ADAMTS5*), and inflammatory (*IL6*) markers were evaluated. Cell viability was evaluated using Live/Dead confocal microscopy. Experiments were performed with three biological replicates and statistical analyses were performed with one-way ANOVA.

RESULTS: Live/Dead staining results showed that most of the cells were alive in both cartilage and synovium tissues after 7 days of culture (Figure 1). GAG release was increased by a factor of 1.5 in tissue cultured in DMEM HG exclusively in the presence of IL-1β and TNF. Although NO release was increased by IL-1β and TNF treatment, there were no differences between samples cultured in DMEM HG and LG media. In cartilage, DMEM HG increased the expression of *ACAN* and *COL2* mRNA compared with DMEM LG. In presence of inflammatory factors, expression of *ACAN*, *COL2*, *ADAMTS4* and *IL6* were higher in samples that were cultured in DMEM HG compared with DMEM LG. In synovium, compared with DMEM LG group, the transcripts levels of *IL6* and ADAMTS5 were increased in samples cultured in DMEM HG in the presence of inflammatory factors. Expression of VEGF mRNA remained unchanged in synovium tissues cultured in different levels of glucose. In subchondral bone, higher levels of glucose increased the expression of COL1 and decreased VEGF mRNA levels.

DISCUSSION: This study revealed that glucose level alters the joint tissue responses to inflammatory factors *ex vivo*. Higher levels of glucose increase the gene expression of both anabolic and catabolic markers, suggesting a key role for glucose metabolism in joint tissues homeostasis *ex vivo*.

SIGNIFICANCE/CLINICAL RELEVANCE: *Ex vivo* co-culture of osteochondral and synovium explants provides a superior condition for mimicking joint and cartilage-related disease mechanisms compared to cartilage monoculture. Optimizing *ex vivo* culture system of these tissues will accelerate the development of novel treatments for joint-related diseases.

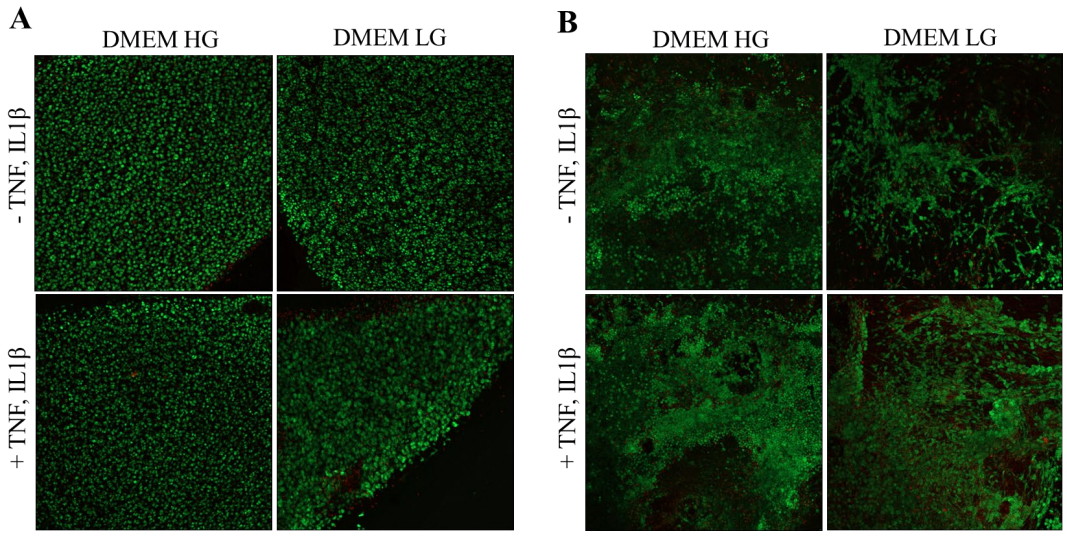

Figure 1: **Live**/**Dead** staining with **calcein** (green) and **ethidium** (red). (A) cartilage and (B) synovium tissues cultured in DMEM HG and LG in absence or presence of inflammatory factors.