SUPPLEMENTARY MATERIAL

Molecular level characterization

Detailed description of qPCR methods

To ascertain whether the different storage conditions produced distinct molecular responses in the cartilage discs, gene expression was analyzed using real-time qPCR. RNA was isolated from the frozen cartilage samples using a modified Biochain Protocol (Cartilage RNA Isolation Kit, K2031010, Biochain Institute, Newark CA). In brief, samples were crushed in aluminum foil with a hammer until a fine powder was achieved. All tissues were maintained in liquid nitrogen during this process to prevent RNA degradation. Using a clean instrument, the powder was transferred to a sterile 1.5 mL tube. Biochain kit solutions (Solution 1, 2, Phenol A) were added to the tissue as directed, with vigorous mixing as required. Chloroform was than mixed and the samples were left for 15 minute incubation on ice and further centrifuged at 18,000g for 15 min at 4°C. The chloroform step was repeated twice to increase the purity of the RNA extracted. Supernatant was then transferred to a new 1.5 mL tube, solution 3 was added, and all contents were mixed well before storage at -20°C for >1 hour. The sample was centrifuged and the supernatant discarded. A 30 uL aliquot of DEPC H_2O/0.1 mM EDTA, along with 15 uL solution 4, were added to the RNA pellet and mixed before storage at -20°C overnight. The following morning, the sample was centrifuged before and after the addition of 1 mL sterile 70% ethanol. The resultant supernatant was discarded and the RNA pellet was dried for 15-20 min before dilution in 50 uL of DEPC H_2O/0.1 mM EDTA and storage at -80°C. Isolated RNA was reverse transcribed into cDNA using the SuperScript III first strand synthesis system (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Gene expression for selected gene markers (Actb, Hprt1, Akt1, Col1a1, Col2a1, Col10a1, Acan, Hapln1, Mfap5, Mmp13, CD14, CD117, CD163, CD4) was quantified using RT-qPCR whereby each reaction was performed with 10 ng/uL of cDNA, QuantiTect SYBR Green PCR kit (Qiagen, Hilden, Germany), and the CFX384 real time system machine (Bio-Rad, Hercules, California, USA). Transcript levels were quantified using the $2^{\Delta\Delta Ct}$ method and normalized to the housekeeping gene Akt1 (set at 100).