Compression-like cell deformation in swelling cartilage

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INTRODUCTION: Cartilage swelling is an indicator of early osteoarthritis (OA) [1]. Cartilage swelling reflects a change in osmolarity of the diseased joint from iso- to hypo-osmotic. An increase in tissue hydration changes the osmotic microenvironment of the residing cells and causes cell swelling. Since the opposing cartilage layers in a joint have different material properties [2], they may swell differently and predispose the more swollen cartilage and cells to mechanical injury. Tissue and cell swelling has traditionally been studied separately [3, 4]. However, the interplay between osmotic-induced deformation of the cell and tissue is not readily apparent. The goal of this study was to synchronously measure tissue and cellular deformation in opposing patellar (PAT) and femoral groove (FG) cartilages exposed to an extreme hypo-osmotic challenge. We hypothesized that the cartilage tissue and the cells would experience tensile swelling strains in all direction during hypo-osmotic loading, and that the soft patellar cartilage would swell more than the stiff FG cartilage.

METHODS: Osteochondral blocks were harvested from the medial FG and the opposing PAT of mature lapine knees (n=6 for FG; n= 6 for PAT). Cartilage tissue and live cells were stained for 1h by 16 µM 5-DTAF and 3 µM Calcein Red/Orange AM, respectively. The stained specimens were fixed in a sample holder and equilibrated in an isotonic saline (315 mOsm) bathing solution at room temperature for 30 min. A three-dimensional (3D) grid pattern with inter-line spacings of 10 µm was imprinted onto a superficial tissue volume of 110 x 110 x 50 µm by photobleaching with a multi-photon laser microscopy (FVMPE-RS model, Olympus) [5]. A 3D reference image of the gridded tissue region was then acquired. Live cells (n=115 for FG; n= 99 for PAT) in the corresponding superficial tissue located at 200 µm to the right of the grid region were also imaged. The bathing solution was changed from isotonic to the limiting hypotonic state of deionized water, after which 3D image stacks of tissue and cells were taken at 1, 3, 5, 25, 40 and 75 min. The 3D images were post-processed for changes in volume and principal strains of the tissue matrix and the cells as a function of time by using the open-source software packages 'lsmgridtrack' and 'resonant_lsm', respectively [6]. A generalized estimating equation (GEE, under Genlin Mixed procedures) available in the commercial software package SPSS was used for statistical analysis.

RESULTS: The tissue matrix and most cells swelled during the hypo-osmotic challenge, but to different extent (tissue: <3%, cells: 11–15%, Fig 1). The volume changes of the tissue and cells were greater for PAT cartilage than for FG cartilage (Fig. 1). For the cartilage tissue, the tissue strains induced by swelling were anisotropic, showing 2–4% stretch and 1–2% compression along the first and third principal directions, respectively. Cells responded differently to the hypo-osmotic challenge than the tissue matrix. First, cell strains were 5–8 times greater than tissue strains (Fig. 1B vs Fig. 1A). Second, the first principal strain direction of the cells was nearly parallel to the articular surface (8–13°, Fig. 1B), as opposed to the 60–61° angle relative to the surface for the tissue. Third, rather than remaining swollen, as observed for the tissue matrix (Fig. 1A), >88% of cells underwent regulatory volume decrease and returned to their pre-osmotic challenge reference volume over time (Fig. 1B). Cell shapes changed in the early phase of swelling but then stayed constant.

DISCUSSION: The novel 3D grid-line imaging of the cartilage tissue [5] allowed for measurements of volume changes in tissue and cells simultaneously. We found that the swelling-induced deformation of the tissue and cells were anisotropic. The tissue matrix increased in volume for the hypo-osmotic condition due to the Donnan effect caused by the proteoglycan fixed charge density. However, the tissue volume increase remained below 3%, likely due to the tension-resistant fibrous network in healthy cartilage. Swelling strains of the *in situ* cells were anisotropic [7], and resembled those found in mechanically-compressed tissue [8] (Fig. 1B). Furthermore, following the initial deformations, cells actively recovered their volume independent of the surrounding tissue strains and they seemed to prioritise volume restoration over shape restoration.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings suggest different mechanisms that cause volume expansion in cartilage tissue and chondrocytes, and they shed light on tissue-cell inter-dependence for changing osmotic environments, such as occur in early OA. These findings foster our understanding of the complex interactions of cell-tissue mechano-transduction in swollen/diseased tissues and are relevant to applications in functional tissue engineering.

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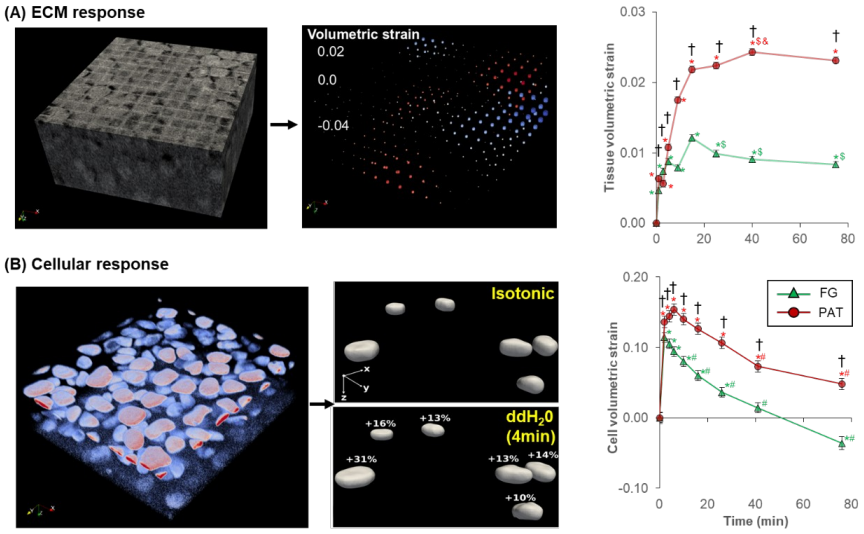

Fig 1. (A) Cartilage tissue and (B) chondrocyte responses to a hypo-osmotic challenge as a function of time. Cells exhibited compression-like deformations in the swollen tissue. The numerical values indicated above individual cells represent the volume change during the hypo-osmotic loading conditions.