A Murine Model of Compression-Induced Ca2+ Signaling by Chondrocytes In Situ

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Introduction: In articular cartilage, Ca2+ signaling plays an important role in the translation of tissue-level forces into chondrocyte activity and may be critical for joint homeostasis. Although research on mechanically-induced Ca2+ signaling has generally focused on chondrocytes in hydrogel constructs, a recent study demonstrated that the response of chondrocytes to load in intact rabbit cartilage differs from their response in 3-D hydrogels [1-3]. Due to the wide availability of transgenic mice that mimic osteoarthritis and other cartilage diseases, a murine model of in situ, compression-induced Ca2+ signaling could serve as a valuable research tool. However, while Ca2+ signaling has been investigated in mouse cartilage as a function of osmotic loading [4], compression-induced Ca2+ signaling is particularly challenging to measure in this thin (maximum thickness~50 μm) tissue and has not been directly characterized. Thus, the aim of this study was to establish a murine model that investigates the effect of static compression on chondrocyte Ca2+ signaling in situ.

Methods: Mouse patella/patellar cartilage complexes (n=9) were carefully dissected and cut into cylinders using a 1 mm diameter biopsy punch. Each specimen was labeled for 1 hour with Fluo-4 AM at 37°C and incubated within phenol red-free DMEM for 1 hour. Fluo-4 AM is a cellular stain that dramatically increases in intensity in the presence of Ca2+. A total of 3 MPa in 1 MPa increments was applied over a period of 30 minutes to each specimen using a microscope-mounted compression device. Chondrocytes with a normalized intensity variance above a critical level were assumed to be exhibiting a Ca2+ transient and the percentage of cells exhibiting transients was computed using a custom MATLAB code. The effect of stress on Ca2+ signaling was analyzed using a repeated measures one-way ANOVA with a Tukey HSD post-hoc test. Significance was set at p<0.05.

Results: Two types of calcium transients were observed: 1) rapid transients characterized by a sudden change in cell intensity and 2) slow transients characterized by a gradual increase or decrease in cell intensity (Figure 1). Slow (type 2) transients were more frequent (data not shown). Within the timescale of our experiments, cell intensities were not found to return to pre-transient levels following a type 2 transient event. The number of cells exhibiting either transient generally decreased at 3 MPa as compared to 1 and 2 MPa (Figure 2) and cells undergoing slow transients were typically located in clusters, suggesting that these events are mediated by local stresses.

Discussion: In this study, a murine model of compression-induced Ca2+ signaling by chondrocytes in their native environment was successfully developed. As in previous studies of intact rabbit cartilage and hydrogel-embedded chondrocytes, Ca2+ signaling was found to be stress-dependent [1-3]. However, to our knowledge, the slow (type 2) transients described in this study have not been observed previously in intact specimens of articular cartilage and may represent a separate signaling pathway from more sudden and shorter calcium cascades. To better understand these potentially novel slow Ca2+ signaling events, ongoing work is aimed at separately quantifying the number of rapid and slow transients.

Significance: Due to the availability of transgenic mice, the model established in this study has the potential to enable comparison of healthy and diseased tissue to help elucidate the role of mechanically-stimulated Ca2+ signaling in cartilage homeostasis.

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Figure 1: Intensity versus time for representative cells exhibiting (a) multiple rapid (type 1) transients after application of 2 MPa at t=0 and (b) a single slow (type 2) transient after application of 1 MPa at t=0. To account for photobleaching and motion out of the focal plane, intensity values at each time point were normalized by the mean intensity of cells that do not exhibit transients.

Figure 2: The mean percentage of cells exhibiting slow transients at 3 MPa was significantly lower than at 1 and 2 MPa. * denotes p<0.05 versus 1 and 2 MPa.

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