Calcium Sources of Spontaneous Calcium Signaling in *in situ* Human Osteoarthritis Chondrocytes

Ying Peng[1], Kaitlyn Dohn[1], X. Lucas Lu[1]
[1]University of Delaware, Newark, DE. Ying Peng: yingpeng@udel.edu

Disclosures: No disclosure

INTRODUCTION: Mechanotransduction plays a critical role in regulating chondrocyte behavior, cartilage development, and osteoarthritis (OA) pathogenesis [1,2]. Intracellular calcium ($[Ca^{2+}]_i$) signaling is one of the earliest responses of chondrocytes under biophysical stimuli. We previously found that chondrocytes in juvenile bovine cartilage have spontaneous $[Ca^{2+}]_i$ signaling similar to active cells such as neurons and cardiomyocytes [3]. The $[Ca^{2+}]_i$ peak has to be initiated by the influx of extracellular Ca^{2+} and could be strengthened by the release of intracellular endoplasmic reticulum (ER) calcium store. Another potential calcium source is its influx through the gap junctions between cells [4,5]. Little is known about the spontaneous $[Ca^{2+}]_i$ signaling of chondrocytes in degenerated human cartilage. This study aims to investigate the spontaneous $[Ca^{2+}]_i$ peaks of *in situ* human OA chondrocytes and the potential signal initiation mechanisms.

METHODS: *Tissue preparation*: Cylindrical cartilage samples (diameter = 3 mm, thickness = 2 mm) were harvested from human knee joints (n = 5, avg. age = 60 years old) from total knee arthroplasty (Fig. 1a) and incubated in chondrogenic medium [6]. Cartilage was divided axially into two halves and treated with 5μM Calbryte-520 (AAT Bioquest) to label the intracellular Ca^{2+}. A half sample was treated with one of the following antagonists for 30 min (n ≥ 3 per group): 1) calcium-free HBSS medium, 2) 10 mM EGTA to chelate Ca^{2+} in both medium and solid matrix, 3) 75 μM 18α-glycyrrhetinic acid (18α-GA) to block the gap junctions, and 4) 11 μM neomycin to inhibit the phospholipase C (PLC) activity that is required for the release of ER calcium store [7]. The other half was used as the corresponding control. *Time-lapse imaging and spatiotemporal parameters of $[Ca^{2+}]_i$ peaks* : Fluorescently labeled cartilage was imaged on a confocal microscope (Zeiss LSM 510) for 30 min with 2-second intervals (Fig. 1b,c). Calcium images were processed with an established protocol to extract the $[Ca^{2+}]_i$ transient of each cell [6]. The responsive rate was defined as the fraction of the number of cells with ≥ 1 $[Ca^{2+}]_i$ peaks over the total number of cells. Spatiotemporal parameters of the $[Ca^{2+}]_i$ peaks, which include the number of multiple peaks, time to reach a peak, time for a peak to relax, magnitude of peaks, and time between two neighboring peaks, were measured on the calcium transient for each responsive cell (Fig. 1d).

RESULTS: Chondrocytes in OA human cartilage showed robust spontaneous $[Ca^{2+}]_i$ peaks. The average responsive rate was 34.6% ± 16% (mean ± SD) for 16 control samples. Among the responsive cells, the average number of $[Ca^{2+}]_i$ peaks was 2.65 ± 2.35 peaks over 30 min. The time to reach a $[Ca^{2+}]_i$ peak from the baseline was 85 ± 67 seconds, and the time between two neighboring peaks was 340 ± 277 seconds. When cartilage was in calcium-free medium, the responsive rate was significantly reduced (9.5% ± 3.2%, $p < 0.05$), associated with lower number of peaks and magnitude of peaks compared to the corresponding control (Fig. 2a). When Ca^{2+} in both medium and cartilage solid matrix were chelated by EGTA, few cells (14 out of 677 cells) had spontaneous $[Ca^{2+}]_i$ signaling (Fig. 2b). With the gap junctions blocked, responsive rate and number of peaks showed no significant changes, but the time to reach a peak, time to relax from a peak, and time between neighboring peaks all increased (Fig. 2c). When the intracellular PLC activity was inhibited by neomycin, the responsive rate significantly reduced (10% ± 14.8% vs 36% ± 4.5% in control, $p < 0.05$). The number of multiple peaks and peak magnitude decreased, while all three of the temporal parameters increased (Fig 2d).

DISCUSSION: This study showed that chondrocytes in OA human cartilage have spontaneous $[Ca^{2+}]_i$ peaks and the responsive rate was similar to the chondrocytes in healthy bovine cartilage [3]. The spontaneous $[Ca^{2+}]_i$ peaks are highly dependent on the influx of extracellular calcium. Due to the negative charges on cartilage proteoglycans, some Ca^{2+} could have be trapped in the solid matrix even when the tissue was in calcium-free medium. This small amount of Ca^{2+} is sufficient to induce $[Ca^{2+}]_i$ peaks in some cells (Fig. 2a). In contrast, when all Ca^{2+} was chelated by EGTA, few chondrocytes had $[Ca^{2+}]_i$ peaks (Fig. 2b). The results of the 18α-GA treatment implies the intercellular gap junction is not a major Ca^{2+} source to initiate spontaneous $[Ca^{2+}]_i$ peaks, but blocking gap junctions could alter the temporal features of $[Ca^{2+}]_i$ peaks (Fig. 2c). Responsive cells can release ATP, which can diffuse to neighboring cells, stimulate the purinergic receptors, and activate the PLC/inositol 1,4,5-trisphosphate pathway to induce ER Ca^{2+} release [8]. As expected, inhibition of PLC activity reduced the responsive rate and significantly changed the spatiotemporal parameters of $[Ca^{2+}]_i$ peaks (Fig. 2d).

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrated the spontaneous $[Ca^{2+}]_i$ signaling in human OA cartilage and revealed the roles of extracellular calcium source, intracellular calcium store, and intercellular gap junctions in spontaneous $[Ca^{2+}]_i$ signaling.

REFERENCES: [1] Lee+ 2017. [2] Lee+ 2021. [3] Zhou+ 2019. [4] Mayan+ 2012. [5] Mayan+ 2013. [6] Zhou+ 2015. [7] Parys+ 2012. [8] Graff+ 2000.

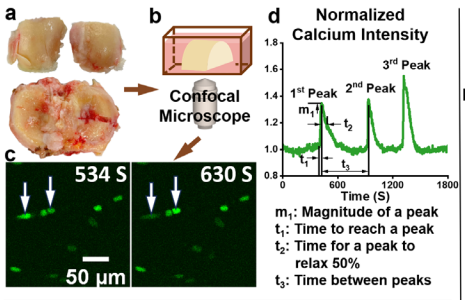

Fig. 1 Spontaneous $[Ca^{2+}]_i$ signaling of chondrocytes. **a)** Cartilage samples were harvested from human knee femoral condyle and tibia plateau. **b)** Confocal imaging. **c)** Fluorescent images show calcium signaling in chondrocytes. **d)** $[Ca^{2+}]_i$ transient of a responsive cell and spatiotemporal parameters.

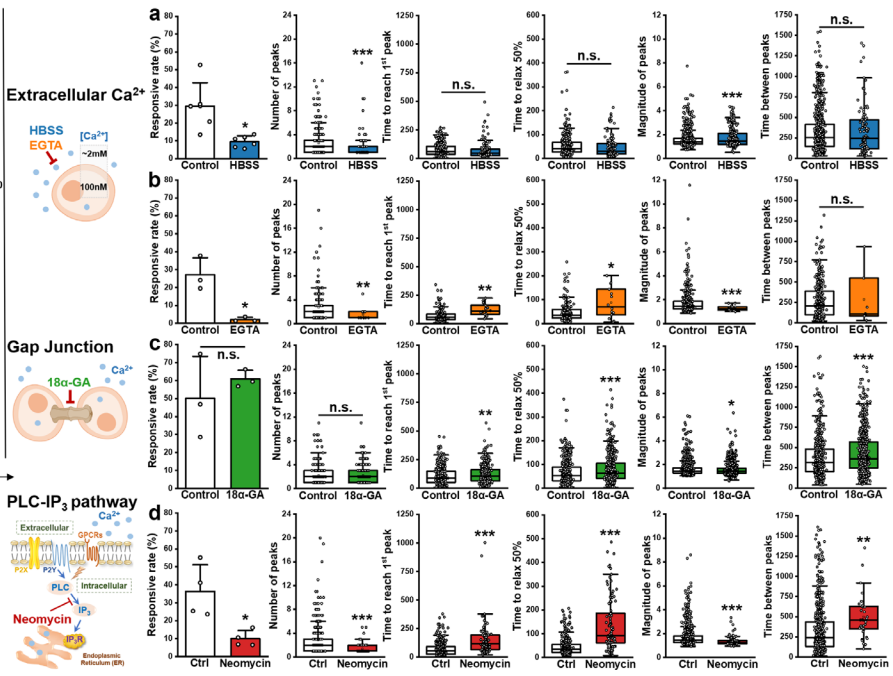

Fig. 2 Spatiotemporal characteristics of spontaneous $[Ca^{2+}]_i$ peaks in antagonist-treated cartilage. a) Calcium-free HBSS medium, **b)** Chelator of Ca^{2+} – EGTA, **c)** Gap junction blocker – 18α-GA, and **d)** Inhibitor of intracellular PLC-IP3 pathway – neomycin (mean ± SD, total number of chondrocytes ≥ 677 from n ≥ 3 cartilage explants/group, *: $p < 0.05$, **: $p < 0.01$, ***: $P < 0.001$, n.s.: not significant).