Hypermetabolic but Non-Proliferative Chondrocytes in Late-Stage Osteoarthritis

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INTRODUCTION: Chondrocytes are the sole cell population in articular cartilage and are responsible for maintaining extracellular matrix homeostasis. During osteoarthritis (OA) initiation and progression, chondrocytes undergo morphological changes, metabolic activity alterations, and aberrant phenotypic shifts [1,2]. However, the altered chondrocyte behaviors in late-stage OA remain incompletely understood. We developed a confocal image-processing protocol, combined with a click chemistry-based assay, to quantify the morphology and matrix synthesis of *in situ* chondrocytes. Using these tools, this study compared metabolic activity, morphology, cell density, and proliferation of *in situ* human chondrocytes in healthy and late-stage OA knee cartilage.

METHODS: *Sample Preparation*: Full-thickness cartilage samples (diameter = 3 mm) were harvested from the knee joints of cadaveric donors (4 males, avg. age = 38 yrs) or late-stage OA patients undergoing total knee replacement (TKR) (2 males, 1 female, avg. age = 68 yrs). *Histology*: Cartilage samples were freshly fixed, paraffin-embedded, and sectioned at 5 µm thickness. Hematoxylin and Eosin (H&E) and Safranin O/Fast Green staining were performed. *GAG/Collagen Labeling and Quantification*: Newly synthesized glycosaminoglycans (GAG) and collagen were metabolically labeled during a 96-hour culture period (sample weight ~8 mg; n = 12/group) using a click chemistry technique (Fig 2) [3]. New GAG or collagen was quantified fluorometrically and normalized to the sample DNA content, measured with Hoechst (Invitrogen™) staining performed in parallel with click labeling. *Confocal Imaging and Cell Morphology Analysis*: Newly synthesized GAG and chondrocyte nuclei were double stained with click chemistry and Hoechst, respectively. Z-stacks of the middle zone cartilage (n ≥ 12/group) were acquired using a Zeiss LSM880 confocal microscope at 0.52 µm intervals (~150 slices). A custom NIH Fiji-based image processing protocol was developed to automatically quantify nuclear, cellular, and nascent GAG volumes. *Chondrocyte Density*: Cell density in full-thickness samples was estimated by the ratio of DNA content to tissue wet weight. In the middle zone, density was further quantified from confocal z-stacks using our image-processing pipeline. *Cell Proliferation*: *In situ* chondrocyte proliferation was assessed using a copper-free click chemistry assay [3]. Samples (n = 6/group) were incubated for 24 h with azide-modified AmdU (30 µM), a thymidine analog, followed by fixation and permeabilization. Incorporated azides on DNA were conjugated with AZ488 DBCO via click reaction, and proliferating cells were imaged on a confocal microscope.

RESULTS: Cartilage from healthy knees showed proteoglycan loss primarily in the superficial zone, whereas TKR samples exhibited extensive GAG depletion and fibrillation in the superficial zone (Fig. 1). Newly synthesized GAG and collagen formed halo-like structures surrounding the chondrocytes, with nascent GAG appearing more intense and tightly attached to the cell membrane (Fig. 2). Compared to healthy cartilage, OA chondrocytes demonstrated significantly elevated synthesis activities, producing 175% more GAG and 97% more collagen during the 96-hour culture (Fig. 3). Consistently, nascent GAG volume in OA cartilage was 21% larger than in healthy tissue (Fig. 5). Cell density was significantly reduced in OA cartilage, by 67% in full-thickness and 17% in the middle zone compared to healthy cartilage (Fig. 4). Imaging analysis further revealed pronounced chondrocyte swelling in OA tissue, with nuclear and cell volumes both 44% larger than in healthy cartilage (Fig. 5). In contrast, proliferative capacity was severely diminished in OA cartilage. While limited proliferation was observed in healthy adult cartilage, virtually no proliferating chondrocytes were detected in late-stage OA tissue (Fig. 6). For reference, using the same protocol, ~50% of chondrocytes in 2-month-old calf cartilage were marked as proliferative.

DISCUSSION: This study provides a detailed comparison of chondrocyte activity in healthy adult versus late-stage OA cartilage. Despite reduced cell density and virtually absent proliferative capacity, OA chondrocytes exhibited markedly elevated GAG and collagen synthesis, consistent with a hypermetabolic, compensatory response to matrix degeneration. The observed cellular and nuclear swelling aligns with prior reports of hypertrophy-like changes [4,5], supporting the notion of a stressed chondrocyte phenotype. In contrast to immature cartilage, where robust proliferation presents, OA cartilage showed virtually no proliferative capacity, implying its limited regenerative potential [3]. Together, these findings indicate that OA progression involves not only matrix loss but also a fundamental phenotypic shift, in which chondrocytes transition from quiescent maintenance to a stressed, metabolically active but non-proliferative state. Therapies that preserve chondrocyte viability or restore proliferative potential may be needed to complement matrix-protective strategies. **SIGNIFICANCE:** Chondrocytes in late-stage OA, though fewer and non-proliferative, display hyperactive metabolic activity, reflecting a paradoxical yet inadequate repair attempt. The methodologies established here offer a new, quantitative pipeline to interrogate chondrocyte behavior in disease and therapeutic contexts.

REFERENCES: [1] Pane + 2023. [2] Boschetti + 2008. [3] Porter + 2022. [4] Lv + 2019. [5] Yamada + 2021.

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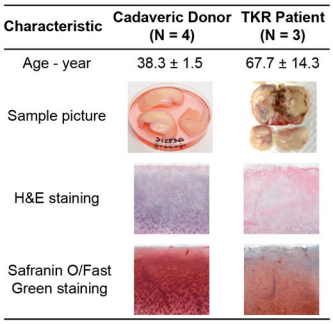


Fig. 1 Picture and histological sections of cartilage from cadaveric donors and TKR patients.

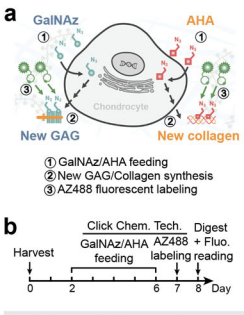


Fig. 2 Nascent GAG and collagen labeling. (a) Schematic of the click chemistry method for labeling nascent GAG and collagen. (b) Timeline for GAG and collagen labeling and quantification. (c) Confocal images of labeled GAG and collagen in cartilage samples.

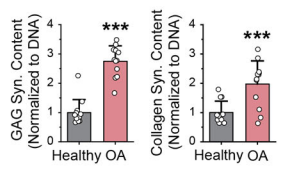


Fig. 3 GAG and collagen synthesis normalized to DNA content. Matrix synthesis was compared between healthy and OA cartilage, revealing that OA chondrocytes showed a higher synthetic capacity.

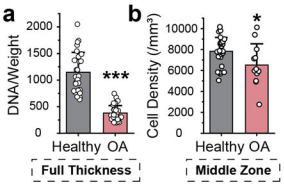
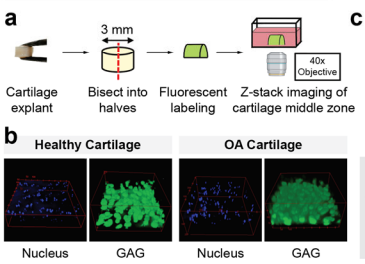


Fig. 4 Chondrocyte density. (a) DNA-to-weight ratio was used as an indicator of cell density in full-thickness cartilage samples. (b) Middle zone cell density was measured from image stacks.



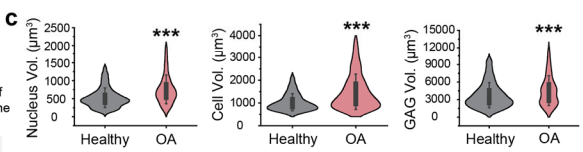


Fig. 5 Confocal imaging, 3D reconstruction, and volume quantification of nuclei, cells, and nascent GAG. (a) Healthy and OA cartilage from the cadaveric donors or TKR patients were fluorescently labeled and imaged by confocal microscopy. (b) 3D reconstruction of confocal z-stack images showing nuclei and nascent GAG. (c) Comparison of nucleus, cell, and GAG volumes between healthy and OA cartilage, with OA chondrocytes exhibiting larger values for all three measures (n ≥ 713 cells/group). *: P < 0.05; **: P < 0.01; ***: P < 0.001

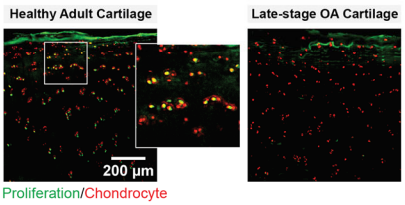


Fig. 6 Chondrocyte proliferation in healthy and late-stage OA cartilage. Limited cell proliferation was detected in healthy adult cartilage, with virtually none observed in late-stage OA cartilage.