Early Clues in the Osteoarthritic Enigma: Multiscale Assessment of Bio-structure-mechanics for Pre-osteoarthritis Detection

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INTRODUCTION: Osteoarthritis (OA) is a degenerative disease that can occur to most articular joints and is most commonly seen in knees. It affects all associated tissues such as the articular cartilage and subchondral bone, which is a leading cause of disability in elderly patients. Currently, there are no effective early clinical diagnostic measures for OA and there lacks disease-modifying drugs from the perspective of treatment except joint replacement surgery. To advance our understanding of what triggers OA and detect the earliest-possible changes, even at the pre-OA stage, are vital to early diagnosis of OA patients and identification of the “window of treatment”. Articular cartilage is mainly composed of type-II collagen. However, type-II collagen fibre diameter is below the optical diffraction limit to be directly resolved by optical microscopy. Recent development in analyzing the polarization-dependency of second-harmonic generation (pSHG) imaging has provided a novel avenue of probing such ultrastructure of cartilage. It has revealed the intricate hierarchical structuring of fibril organization at a few hundred nanometers and intrabilateral organization at the molecular scale in cartilage, which were found sensitive to mechanical loading. It is hypothesized that this multiscale structure organization is crucial to micromechanical functions and is associated with the early progression of osteoarthritis (OA). In this study, we will investigate the biology-structure-mechanics relationship of cartilage in the rat medial meniscus transection (MMDT) model at various post-operative OA time points. The choice of the model is primarily employed to evaluate preventive therapies, and we aim to mimic clinical OA patients ranging from pre-grade 1 to grade 3. This project incorporates biological assessments, nonlinear biophysical imaging, and nanoindentation to furnish a comprehensive understanding of the impact and potential biomarkers of OA progression and clinical application.

METHODS: All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of the Tokyo Medical and Dental University in Japan (protocol number: A2022-028C5). The OA surgery procedure, which involves a complete incision of the meniscus at its narrowest section, was conducted at 8-week-old SD female rats which is to destabilize the rat’s knee joint, effectively simulating osteoarthritis pathology. The changes in articular cartilage and subchondral bone were evaluated by histology, micro-CT, pSHG, and nanoindentation at pre-OA (1 week), early (3 weeks), mid (6 weeks) post-surgery stages in this model, intact rats without surgery as the control in this study. The longitudinal cross-section plane of the medial condyle of the rat tibia (from articular surface to trabecular bone) was imaged using pSHG with a pixel resolution of 1 um. The pixel-based SHG signal intensity at corresponding polarization angles was fitted by the circular function to derive the degree of alignment (Er) of collagen fibrils. To obtain the cartilage mechanical properties (i.e., the fibrous network with substance phase of the matrix), the medial side of the fresh tibia at every time point post-surgery was prepared for nanoindentation tests. During the nanoindentation tests, the specimens were kept moisturized with PBS. A flat-end indenter with a diameter of 10 um at a loading rate of 500nN/s, with maximum forces of 50uN was used to measure the instantaneous modulus of the cartilage tissue (E1) at ROI1 (medial 1/3) and ROI2 (middle medial) on the medial side. From the time-displacement curve of instantaneous unloading, after curve fitting, two Er values were derived. One is reduced modulus (E1) High, which when curve fitting results in the R-square value of 0.99, will yield a higher Er value representing the material’s stiffness before any time-dependent or viscoelastic effects. The other is E1 Low, defined during curve fitting where the R-square value is at its minimum but greater than 0.8. This will produce a lower Er value, indicative of the influence of viscoelasticity. Differences for all data were evaluated by a two-tailed Student’s t-test. Significance was set at p < 0.05.

RESULTS SECTION: The rats after 3 weeks of surgery displayed cartilage degradation and proteoglycan loss evidently. The parameter of subchondral bone density (p<0.05) was found even from the 3rd week post-surgery and then increased again by the 6th week. Interestingly, dramatic changes in Er were observed as early as 1-week post-surgery in the nanoindentation tests. In both ROIs, Er High significantly decreased (p<0.05), while Er Low showed a similar trend but without statistical significance. This indicates that the micro-scale mechanical properties of the cartilage had already undergone changes even in the pre-OA stage, especially in ROI1. As OA progressed by the 6th week (mid-stage OA) the cartilage became even more stiffened compared to 3 weeks, particularly in ROI2 (p<0.05), even surpassing the control levels.

DISCUSSION: In our study, we observed early damage in cartilage microstructure and mechanical properties. The ultrastructure features characterized by pSHG exhibit high sensitivity to quantify early OA and its progress. The decreased alignment of collagen fibrils in both articular cartilage and subchondral bone was found even from pre-OA which indicates the crosstall between cartilage and bone might already been triggered in pre-OA. Regarding the mechanics of cartilage, as the decreased quality of alignment of collagen fibril, the loss in mechanical properties at a very early stage was also observed. However, the mid-stage stiffening might foretell cartilage loss due to tissue degradation, especially in non-mineralized cartilage, leading to tests on the area of calcified cartilage. Moreover, the distinct alterations displayed by Er High and Er Low indicate that viscoelasticity might contribute to the mechanical properties of cartilage. This is consistent with the fact that cartilage contains a large part of water and macromolecules, suggesting the need for us to challenge developing a viscoelastic load function for biological tissues. In summary, during the pre-OA phase, we detected changes in joint cartilage collagen arrangement and mechanical properties, which could potentially serve as a prospective biomarker.

SIGNIFICANCE/CLINICAL RELEVANCE: This study’s findings highlight the potential of early microstructural and mechanical changes in joint cartilage, detected using advanced techniques, as pivotal markers for the early diagnosis and intervention of osteoarthritis for patients.

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