The Correlation Of Cartilage Damage With Mechanical Properties In Early Stages Of Osteoarthritis Using A Mechanical Loaded Mouse Model

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Introduction: Osteoarthritis (OA) is a multifactorial disease affecting both the cartilage and the subchondral bone. Progressive degeneration of cartilage and subchondral bone deteriorates its mechanical stability [1]. As such, development of an early diagnostic method of OA is critical to prevent the loss of the load bearing ability of the OA joint. In our previous study, we found that damaged cartilage targeted with monoclonal antibodies to type II collagen (MabCII) and conjugated to near infrared fluorescent dye (NIF) can be readily detected and quantitated in the damaged knees of mice using the IVIS imaging system (PerkinElmer, MA USA) [2]. Correlation between IVIS measurements of fluorescence intensity and histological damage in mechanically loaded mouse knees suggested that this non-invasive method for diagnosis of early stages of cartilage injury and posttraumatic osteoarthritis (PTOA) could conceivably be used as a targeted detection and delivery system. However, the IVIS imaging reveals cartilage surface changes only, thus there is a need for another diagnostic method that can be used to detect changes in the cartilage-subchondral bone complex in correlation with the IVIS imaging system. The association between changes of IVIS measurements and mechanical properties at the damaged cartilage has not been evaluated.

To address this concern, we investigated the change of mechanical properties in the cartilage-subchondral bone complex of the mechanically loaded mouse model using dynamic mechanical analysis (DMA) and compared the change in fluorescent intensity using the IVIS imaging system in cartilage degeneration.

Methods: The mechanically loaded knee model of Poulet [3] was used to investigate the changes in mechanical properties of articular cartilage and subchondral bone in the mouse knee joint in C57Bl/6 mice (n=18).

Mechanical Loading for PTOA; To simulate PTOA development, 160 cycles of 9 N compressive loading of one knee joint were administered three times weekly over a period of two weeks. In order to make a comparison between direct traumatic damage and subsequent PTOA, two experimental groups were set up. Group 1 received two weeks loading to mimic a traumatic condition (Trauma) and those in Group 2 were loaded for two weeks and then maintained for an additional three weeks after the loading to allow time for disruptive metabolism to progress (PTOA). A group of mice that were not loaded were set as controls (normal).

Mechanical loading methods were adapted from Poulet’s protocol. Mice at 8-9 weeks of age of equivalent weight (<10% variance) are anesthetized with 4% Isofluorane. The left leg of each mouse was positioned within the ElectroForce 3200 electromagnetic testing system: the proximal tibia rested in the upper cup with the dorsiflexed ankle inserted into the bottom cup. A cycle of compressive axial loads
applied 9N of force 160 cycles over a period. A static offset load of 2N is used to maintain contact between the specimen and the fixture.

Treatment and Imaging: Mice were injected retro-orbitally with 100µl of XenoFluorTM680 labeled monoclonal antibody specific for type II collagen. After 24 hours, the mice were anesthetized and IVIS® scanned for the dye. The mice were then sacrificed, and their legs were stored in a freezer at -40°C until they were required for further testing. Freezing has been previously shown not to change the mechanical properties of articular cartilage [4].

DMA Analysis: After thawing and removal of soft tissues, the whole tibia was glued on the jig of a loading machine. Preloading (1N) was utilized to secure the specimen on the loading jig. Then, DMA was performed by applying compressive cyclic displacement (0.01 mm ± 0.0025 mm) at the range of 0.5 to 3 Hz following the established protocol [4, 5, 6]. The corresponding force ranged between 0 and 0.5 N. Displacement was controlled by a transducer with 15 nm resolution. The cyclic force and displacement was measured to obtain dynamic elastic (storage) (K’') and viscous (loss) (K'') stiffness. Dynamic complex stiffness (K*) was computed with an equation of K*= K’+iK’’ (Fig. 2). Viscoelastic tangent delta (tan δ), which represent ability of loading energy dissipation, was computed by K’’/K’.

Statistics: All experiments were performed independently at least three times. A student’s t-test and analysis of variance were used to determine statistical significance. A p-value of less than 0.05 was considered statistically significant.

Results: IVIS scanning was used to show that NIF-MabCII bound preferentially to damaged cartilage in vivo. Figures 1 show the targeted fluorescence localizing to the loaded left knee and the cartilage defect was not recovered after 3 weeks suggesting the development of osteoarthritis. The ROI further supports the selective binding of the MabCII-NIF by showing an increased intensity signal in the loaded knee compared to the unloaded knee (Figure 1B). In figure 2, the results showing the change of mechanical property in whole cartilage-bone complex. The data shows that difference of dynamic stiffness and tan δ between mechanical loaded knee vs contralateral knee. The traumatic injury increased the difference of viscoelastic tan δ 3.2 times but the dynamic stiffness did not change. After 3 weeks in the PTOA group, the difference of viscoelastic tan δ increased 2.75 times and the dynamic stiffness increased 2.25 times.(Figure 2)

Discussion: Studies on the pathologic events of OA have been motivated by the use of animal models, which may elucidate the complex inter-relationship between cartilage degeneration and subchondral bone change. Combining data from the IVIS scan for CII targeted fluorescence and the change of mechanical properties of cartilage-bone complex in mechanically loaded mouse knee by DMA revealed cartilage damage is accompanied with changes of dynamic stiffness and loading energy dissipation of the complex. The results also suggest that these changes may affect the damage risk of the subchondral bone causing traumatic injury to develop to the OA at long last in life. Earlier detection of cartilage damage and more effective interventions are needed for individuals with OA to avoid chronic pain and costly future treatments. The current findings will be useful as a monitoring tool for studying the progression of OA and the effectiveness of intervening treatments with pharmacological or biological agents.

Significance: This study shows that as the degree of fluorescence changes, measured by IVIS imaging, so does the DMA value reflect change in the mechanical properties of the cartilage-subchondral bone complex in the PTOA mouse model.
Figure 1

(A) IVS scan image after 24 hours injection of MenB oligofragment into normal mouse, trauma and PTOA induced mouse. Trauma induced by 6 times mechanical loading during 2 weeks and PTOA group maintained for an additional three weeks after the loading to allow time for disruptive metabolites to progress. (B) ROI of Radiant efficiency (RK: Right knee, LK: Left knee) (Asterisks indicate statistical significance with p < 0.05 (*), n=6 per group).

Figure 2

(A) DMA analysis: Difference of viscoelastic tan delta (B) (A) and dynamic stiffness on mechanical loaded knee vs contralateral knee. (B).

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