The Pathology of the Calcified Zone of Articular Cartilage in Post-traumatic Osteoarthritis in Rat Knees


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Introduction: The calcified zone of cartilage (CZC), where cartilage matrix is calcified, is separated from the other zones of cartilage by the tidemark. The CZC borders the subchondral bone with the cement line. Matrix mineralization in CZC allows gradual transition of mechanical properties between cartilage and bone. It would become a focal zone, however, during the development of posttraumatic osteoarthritis (PTOA) whose pathology features mechanical disorders of the joint. In this context, changes in CZC are expected but there are few investigations focused on the CZC in PTOA. In this study, PTOA pathology, CZC areas and tidemark roughness at tibial plateaus in rats were quantified by histomorphometry.

Methods: 1. Animal model of PTOA: Ten male Wistar rats, 12 weeks of age, were evenly divided into control and experimental groups (approved by Institutional Animal Care and Usage Committee). Under anesthesia, the ACL was transected and the meniscus was resected in both knees of rats. Sham surgery was performed on the rat knees in the control group and consisted of opening the joint capsule but no ACL transection and meniscectomy. The operated limbs were not immobilized and rats were allowed to access food and water ad lib. Rats were euthanized four weeks post-operatively. A total of 19 knees (one in the experimental group was eliminated due to infection) were disarticulated and the tibias were coronally bisected, using a custom made jig for accurate positioning and reproducible sections. Tissue sections of upper tibias were cut and stained with Safranin-O, Fast Green, and Hematoxylin.

2. Image analyses: For histological analysis, each medial and lateral tibial plateau was divided into three areas: medial (closest to the tibial spine), central (center of the tibial fossa), and peripheral (closest to the edges of tibial plateau, Fig 1A). An equally sized, consistently located portion from each third of the medial or lateral tibial plateau was selected for histomorphometry. From the same specimen, the same locations on two or more consecutive sections were analyzed and averaged in order to reduce systematic errors. On each selected imaging area, the degree of PTOA, CZC area and the tidemark roughness were quantified (Fig 2B. white arrows: tidemark; black arrows: straight line for calculation of tidemark roughness; open arrows: cement line). The Mankin’s score was used to assess the severity of cartilage degeneration.

Results: 1. Cartilage degeneration in PTOA joints: The pathology of PTOA induced by meniscectomy and transaction of ACL included erosion of cartilage surface, loss of proteoglycan from the matrix, formation of chondrocyte clusters. The Mankin’s score in the experimental group showed significant cartilage degeneration at all three selected locations of the medial plateau and the peripheral portion of the corresponding locations in the control group.

2. PTOA associated CZC pathology: The CZC area varied significantly among the six defined locations across the tibial plateau. Within the control group, the CZC area of the central area of the medial plateau was the smallest and the medial area of the lateral plateau was the largest. Among several locations, the CZC areas were statistically different, such as medial versus lateral plateau in the central and peripheral areas; the medial versus central area in the medial plateau. A significant change in the PTOA knees was the disappearance of statistical difference in the CZC area among many locations across the tibial plateau. The remaining differences between CZC areas in the PTOA knees were the medical area of the lateral plateau versus the peripheral and central areas of the medical plateau, which were the same as in the control. In the PTOA knees, the smallest CZC area was in the peripheral area of the medial plateau, whereas the largest was, the same as the control, at the medial area of the lateral plateau. Each CZC area of the six locations on the tibial plateau in the PTOA joints did not differ significantly from the corresponding locations in the control group, although four of the six areas on the tibial plateau in the PTOA joints showed a tendency of reduced CZC area.

The tidemark roughness was not equal across the tibial plateau in the control group. The lowest tidemark roughness was in the peripheral area of the medical plateau. There were significant differences in the tidemark roughness between several pairs of locations, such as the peripheral area versus medial and central areas on the medial plateau and the central area of the lateral plateau. The differences of tidemark roughness among the six defined locations on the tibial plateau, however, did not exist in the PTOA knees. Compared with the control, tidemark roughness in the PTOA knees was reduced in general, except at the peripheral area of the lateral plateau. When the tidemark roughness was compared between the PTOA knees and the control at corresponding locations, it was found that tidemark roughness was significantly reduced in the PTOA joints at the central and medial areas of the medial plateau.

3. Correlation among PTOA pathology, CZC area and tidemark roughness: No correlation was found between the Mankin’s scores
and CZC areas. The Mankin’s scores and tidemark roughness in the PTOA joints were inversely correlated \( (r = -0.65; p = 0.02; \text{Fig 2}) \).

**Discussion:** This study employed a model of acute joint injury leading to accelerated joint degeneration. PTOA developed in all three selected locations on the medial plateau and the peripheral portion of the lateral plateau, but the central and medial portions of the lateral plateau were spared from significant cartilage degeneration. The severity of PTOA was the greatest in the peripheral portion of the medial plateau, followed by the central portion of the medial plateau.

CZC thickness (area) varies topographically in the same joint, and this was true in the control rat knees. This topographical variation of the CZC area on the same tibial plateau, however, was minimized in the PTOA joints---only 2 paired locations were statistically different. It is most likely that the changes of topographic patterns were responses of the CZC to the pathology and biomechanic alterations in the PTOA joints. Topographically, the tidemark roughness varied between a few pairs of locations on the tibia plateau in the control joints. In the PTOA joints, however, no differences were found between any pair of locations on the tibial plateau. Overall, the tidemark roughness decreased in the PTOA joints. The tidemark roughness was significantly reduced at the central and medial areas of the medial plateau in the PTOA joints, when compared with the control joints. Importantly, the reduced tidemark roughness was inversely correlated with the severity of cartilage degeneration. In PTOA joints, both the central and medial portions of the medial plateau, where had reduced tidemark roughness, showed the largest increases in Mankin’s score. The peripheral portion of the lateral plateau had increased tidemark roughness in the PTOA joints, but it incurred the smallest change in Mankin’s score. In summary, tidemark roughness is locally reduced in PTOA induced by ACL transection and medial meniscectomy and this is inversely correlated with Mankin’s score.

**Significance:** The CZC pathologies discovered in this study provide insight to the progression of PTOA and are important to differentiate PTOA from primary OA.

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