Disease Progression and Pain Vary due to Osteochondral Defect Location in a Rat Model

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Introduction: Preclinical models of osteochondral defects (OCDs) are fundamental test beds to develop and evaluate novel treatment modalities prior to clinical translation. To increase the rigor and reproducibility of translational science and to ensure a robust "go or no-go" of products, we must thoroughly evaluate and establish these preclinical models. Specifically, the location of the osteochondral defect can impact disease progression, pain, patient mobility, and treatment outcomes. As we appreciate that osteoarthritis (OA) is a whole joint disease that impacts patient pain and function, we must evaluate the preclinical models beyond assessment of just the defect region. The objective of this study was to compare the whole joint disease progression and pain phenotype between two OCD models in rats that differed in the surgical approach and the location of the defect. We hypothesized that a weight-bearing OCD would result in greater tissue degradation and pain than an OCD located in the trochlear groove of the femur.

Methods: *Surgical Approach:* Male Lewis rats (12-weeks old) were anesthetized with isoflurane and their left hind limbs were prepped under sterile conditions. We used a medial parapatellar approach for the trochlear OCD (#1). In this approach, a median skin incision was made over the patella, followed by a median parapatellar arthrotomy (Fig.1A). The patella was dislocated to access the trochlea and the osteochondral defect was created 0.8mm deep into the distal trochlea (towards lateral side) using a 1.5mm spherical burr under continuous saline irrigation. The OCD was flushed with saline to free debris, and fibrin glue was placed into the defect area. The patella was repositioned, and the quadriceps tendon was sutured with interrupted 4.0 Vicryl sutures and skin closure was performed with staples. For the weight-bearing condylar OCD (#2), a modified sub-vastus approach was employed. Briefly, the extensor mechanism was spared with no dislocation of the patella. After a midline skin incision, an inverse-L shaped arthrotomy was made with a first medial parapatellar cut and then a second cut parallel and proximal to the joint line (Fig. 1B). The OCD was created using the same burr technique as the first approach but in the weight-bearing area of the medial femoral condyle. OCD was flushed with saline, and fibrin glue was used to fill the defect area before closure of the surgical site. Corresponding sham surgeries were performed and used as control. All rats were allowed to move freely for 8 weeks post-operatively. All studies herein were approved by IACUC. *Phenotypic Assessments:* Mechanical (secondary) allodynia and spontaneous gait were assessed longitudinally every two weeks until the study ended. Spatiotemporal measures of gait were assessed using the Experimental Dynamic Gait Arena for Rodents (EDGAR) and were corrected for rodent mass and velocity using a healthy database. We used a partial least squares regression (PLSR) to reduce the multivariate gait data to three latent variables and to discriminate unique gait patterns between the two models. Macroscopic pictures of the rat knee joints were taken to observe signs of cartilage degeneration. Contrast enhanced micro-computed tomography (EPIC-µCT) images were acquired to evaluate 3-dimensional aspects of the articular cartilage, defect area and meniscal ossicles. Histological sectioning and staining for SafO, Toluidine blue, collagen type I and II were also performed.

Results: OCDs were evident macroscopically on the trochlea and the medial condyle at 8 weeks post-surgery for Approach #1 and #2, respectively. From the macroscopic view, osteochondral defect area was slightly depressed, not smooth and covered with repair tissue. The medial parapatellar approach #1 showed signs of cartilage degeneration throughout the knee joint but was most severe in the femoral-patellar joint. Swollen cartilage with osteophytes were observed in the trochlea of some samples. Cartilage swelling was also found in both the femoral condyles and tibial plateaus. In contrast, the minimal invasive approach #2 only showed cartilage swelling in the medial femorotibial compartment. The femoral-patellar joint showed no signs of degeneration. Swollen cartilage was only seen in the medial femoral condyle and medial tibial plateau while the lateral femorotibial compartment did not show any signs of degeneration. At eight weeks, the defect area was filled with repair tissue and lacked SafO staining, consistent with the lack of collagen type II expression. The subchondral bone was partially reconstituted 8 weeks post-surgery. Using EPIC-µCT, OCDs were recognizable and subchondral bone changes were evident. The medial parapatellar approach #1 did not affect the ossicles while approach #2 significantly affected the mineral density and the ratio of bone surface (BS) to bone volume (BV) of the medial meniscal ossicle (Fig. 1G, I). For pain assessment, we did not observe any allodynic pain phenotype in either model. The two models presented with unique gait strategies, as indicated by a separation along the first latent variable (Fig. 1J). The four parameters that contributed the most to this separation were: imbalance on time spent between the two hind limbs, the relative timing and length of steps in the hindlimbs, and hind step width. Further univariate analyses confirmed an increase in the duty factor imbalance in the trochlear OCD model. These results demonstrate that rats that received a trochlear OCD shortened the step length of the affected limb, whereas those that received a condylar OCD compensated by unloading the affected limb, shifting to more time on the contralateral limb, and by narrowing their step width.

Discussion: Osteoarthritis was evident in both rat OCD models. However, approach #2 limited OA to the defect location while approach #1 exhibited whole joint OA. While the surgical approach did not seem to affect the degree of degeneration, the location of the defect was critical in dictating the severity of OA. In approach #2, loss of bone mineral density in the ossicle may indicate degeneration of the meniscus, which suggests more advanced OA. Surprisingly, we did not observe allodynia in either OCD model. Clinical healing after microfracture can mitigate short-term pain; so, early spontaneous healing with inferior tissue, such as in these OCD models, may be sufficient to delay pain onset. We observed functional differences in gait between the two models. Interestingly, these gait modifications may be associated with morphological changes. In the condylar OCD model, a flattening and demineralization of the medial ossicle was found. Such a change could drive a shift toward knee adduction, and naturally bring the feet closer together even in a neutral stance. With trochlear OCD, dislocating the patella may cause patellar mal-tracking and pain in high knee flexion. Thus, the rat may have shortened step length due to reduction of flexion/extension range of motion.

Significance: Creating an osteochondral defect in a rat knee affects the whole joint. Depending on the location, trochlea or femoral condyle, the severity of OA and gait function differs. The effect of weightbearing should be considered when assessing cell and growth factor therapies for OCD.

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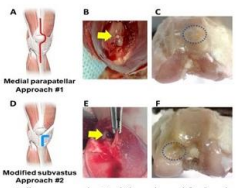
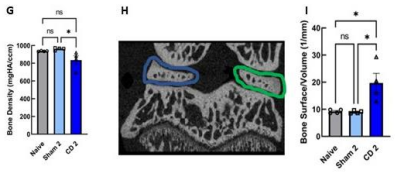
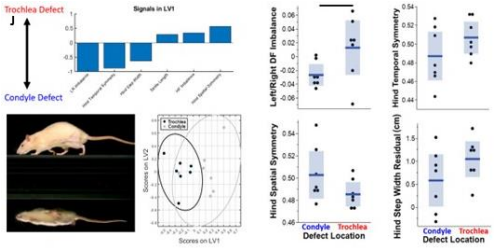


Medial parapatellar approach #1 (A) and modified sub-vastus approach #2 (D) performed on Lewis rats. Chondral defect (yellow arrow) was placed in the trochlea (B) or medial femoral condyle (E). Femurs (C, F) and defect (blue circle) were analyzed 8wk post-surgery.



Chondral defect decreased the bone mineral density of meniscal ossicle only for approach #2. Middle image represents micro-CT image of rat knee joint, blue contour was lateral ossicle and green contour was medial ossicle (H). Bone mineral density (G) and specific bone surface for medial meniscal ossicles (I). * represents $p<0.05$; ns is 'not significant.'



Defect location affects the gait (K).