signal producing segment and two control segments for robustness. To validate our method, we applied it to naïve murine knee joints and extracted the naïve chondrocyte transcriptome.
Methods: Six knee joints were collected from male C57BL/6J mice aged 12 weeks (n = 3). After preparation, each femoral condyle was cut into 3 different segments (18 tissue segments in total) which were defined as follows: F1 included the articular surface, subchondral bone and a small growth plate area of the femur (lateral and medial condyles), F2 included growth plate, bone marrow, non-loaded articular cartilage (patella) and bone while F3 comprised bone marrow and bone. The cutting procedure was verified via histology (H&E- and Safranin-O staining) and weight control. Segment samples were transferred to RNAlater (Qiagen), frozen at -80°C and cyro-pulverized. RNA was extracted via Trizol and chloroform extraction with subsequent purification via the RNeasy Mini Kit (Qiagen). DNA was analysed in the residual material after RNA isolation to get an overview of the amount of cells in the different segments. Purity of the RNA was analysed via Nanodrop and RNA integrity and quality was verified via Bioanalyzer. RNA was processed and applied to microarray chips (18x Affymetrix Mouse Gene Chips 430 2.0).
Results: Upon inspecting the H&E- and the Safranin-O staining, segments F1 and F2 were seen to contain cartilage, whereas F3 did not. Hence, F3 was used as a control against F2 and F1. In the naïve model, F1 and F2 cartilage can be considered to be load bearing and non-load bearing cartilage, respectively. Therefore, F2 was used as the control of non-weight bearing cartilage to F1. We obtained a mean amount of 8,590 ng (min: 2,772 ng - max: 16,023 ng) total RNA of good quality (mean RIN: 8.8; min: 7.5 - max: 9.5). There were 121 F1 genes and 68 F2 genes differentially expressed to F3, respectively. Nearly 90% of these genes were found to have a functional annotation (signaling proteins, glycoproteins, disulfide bonding proteins, or secreted proteins), and their classification had a Benjamini-Hochberg corrected *p-value* of less than *0.05*. The F2 genes differentially expressed to F3 were contained in the set of differentially expressed genes between F1 and F3. Thus, F1 and F2 have a similar differentially expressed gene set with respect to F3, where in F1 slightly more glycoproteins and disulfide bonding proteins were expressed than F2.
Conclusions: The technique of delineating the chondrocyte transcriptome from multiple cartilage sections is promising. In contrast to studies from the literature, we aimed at establishing a protocol which neither uses the whole joint nor pools extracted cartilage samples in order to avoid the loss of valuable information. In the naïve case, where the differential expression between tissues in the knee is known to not be vastly different, the method was still able to detect differences between bone, non-loaded cartilage, and loaded cartilage. Furthermore, the method is robust to cutting variation, and if applied to a disease model, the use of two control segments (non-loaded cartilage F2 and bone F3) will prove effective at amplifying the disease phenotype in the F1 segment.

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THE SUPERFICIAL ZONE IN CARTILAGE ADAPTS WITH STIFFENING WHEN CHALLENGED TRIBOLOGICALLY

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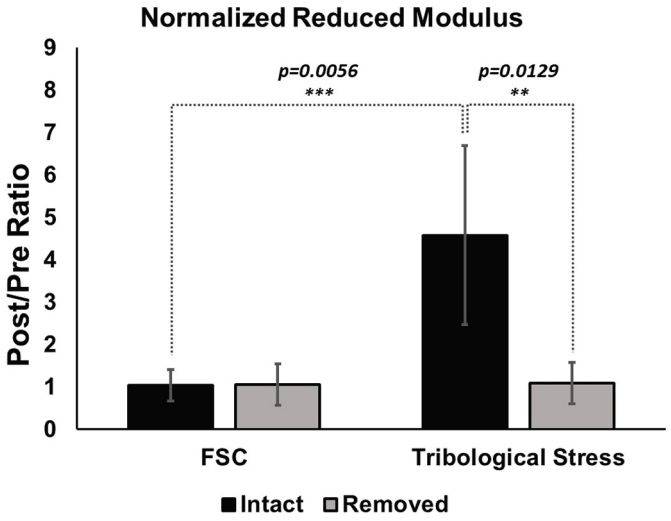
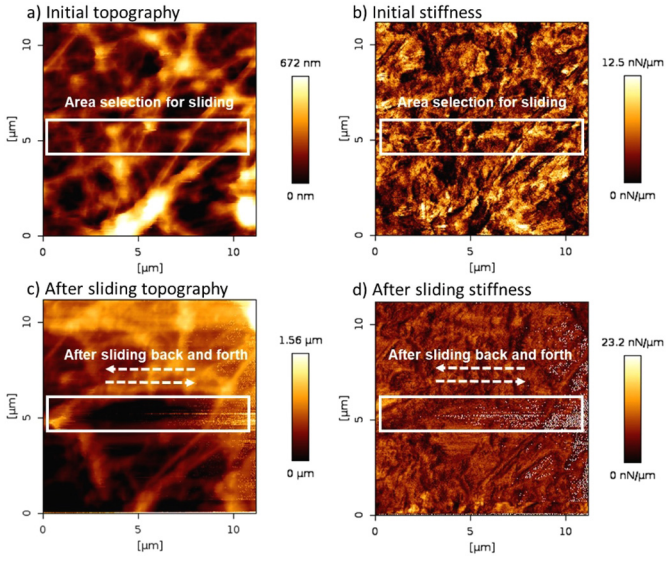
Purpose: In osteoarthritis, the superficial zone of articular cartilage is compromised by fissures and cracks and, thus, may lose its intended function. While there is agreement that the superficial zone is important for the load bearing capacity of the tissue, its mechanical response upon articulation is less investigated. In previous studies, using fully intact cartilage samples, we have shown that the cartilage surface undergoes a transient stiffening response upon articulation. Here, we hypothesize that this response is lost when the surface is removed. In order to address this question we used a tribological bioreactor and articulated on cartilage samples with and without superficial zone. Further, we started to study structural changes at the surface with an atomic force microscope (AFM) before and after the application of tribological stress.
Methods: Twenty-four 14x20x3 mm oval bovine cartilage explants that had undergone one freeze-thaw cycle were distributed into four groups per animal: (1) intact surface and articular loading; (2) removed surface and articular loading; (3) intact free-swelling-control (FSC); 4) removed surface FSC. Thus, each group had n=6 samples. A vibratome was used to remove the superficial zone of the explants (at least 20% of full sample thickness). Microindentation stress-relaxation tests were performed in the center of explants using a Hysitron TI 950 with a 20 μm spherical indenter and an 8 μm indent depth. All indentation on explants was performed submerged in 1× PBS to maintain tissue hydration. Following initial characterization, cartilage explants were removed from the indentation sample holder and placed into the bioreactor. Cartilage explants were confined in porous polyethylene scaffold rings and fixed in a well. Using a 32-mm ceramic ball (Ra ~10 nm), a 40 N static confined compression was applied that lead to approx. 2 MPa contact stress. The ball oscillated at 0.5 Hz, and was migrated at a speed of 1mm/s across the cartilage surface generating a wear scar length of 10 mm. Following 1-hr of bioreactor testing, microindentation was repeated in the center/wear scar. The reduced modulus was obtained using the Oliver-Pharr method on 80-95% of the unloading curve. Cartilage surfaces removed by vibratome were prepared for AFM analysis (Nanowizard Ultra, JPK Instruments, Germany). All measurements were conducted in 1× PBS. Using QI imaging, a force sampling mode, an initial image of 256 x 256 pixels (approx. 10 x 10 μm^2) was obtained. The AFM was then put into contact mode, and an 80 nm silicon tip was repeatedly (>850 times) slid linearly across the cartilage surface. Following these sliding experiments, the tissue was reimaged in QI mode. For data analysis, the ratio of post- to pre-bioreactor reduced modulus was calculated and analyzed using Shapiro-Wilks tests for normality and Paired Student's T-tests.
Results: Our results show that intact surface groups exhibited significantly increased stiffening following articular loading compared to





the FSC (p=0.006). For the removed surface groups, no significant difference between articulated and FSC explants was detected (p=0.927) (Fig 1). Also in the AFM the cartilage surface demonstrated topographical and stiffness related changes upon sliding that are shown in Figure 2.

Conclusions: Surface stiffening following tribological loading may be an important biomechanical function of load transfer during articulation. Surface removal leads to a loss of this adaptive response. The stiffening response may be related to a reorganization of matrix molecules that are unique to the superficial zone. In the future we plan to investigate the influencing parameters of this stiffening response and identify the matrix molecules that are involved.

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EVALUATING PHARMACOLOGICAL INHIBITION OF PPARDELTA IN A RAT MODEL OF POST-TRAUMATIC OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is a painful chronic disease affecting all joint tissues. Previous studies in our lab have demonstrated that nuclear receptors are involved in regulation of cartilage homeostasis. In particular, activation of nuclear receptor PPARdelta in-vitro results in the upregulation of genes coding for fatty-acids and cartilage-matrix degrading proteases. We have also shown that genetic inactivation of PPARdelta specifically in cartilage can be protective in the DMM model of post-traumatic osteoarthritis in mice (Ratneswaran et al, Arth Rheum 2015), thus illustrating it could be a potential therapeutic target. In order to establish the viability of PPARdelta inhibition as a pharmacologic therapy for OA, we conducted a pilot study (N=5) using two pharmacological PPARdelta inhibitors in a rat model of post-traumatic OA. Our pilot study indicated that 4 weeks post-surgery rats treated PPARdelta inhibitors did not experience the same pain-like behaviour (spontaneous activity, weight-bearing) as vehicle-control treated rats. Structural protection to the cartilage and underlying bone had trended towards protection but was not statistically significant. Our current study is an extension of the original pilot, examining pharmacological PPARdelta inhibition in rat post-traumatic OA. We hypothesize that inhibition of PPARdelta will delay the progression of post-traumatic OA in animal models, and that rats treated with PPARdelta inhibitors will present with less pain like behaviour.

Methods: In our first cohort, 300-350g male Sprague-Dawley rats (n=5 /group) were subjected to sham control surgery or an anterior cruciate ligament transection (ACLT)/partial medial meniscectomy (PMMx) surgery and were systemically treated with two different inhibitors of PPARdelta (GSK0660, 3787) or vehicle control DMSO for six days per week, starting one day post-surgery, for four weeks. Weekly testing for spontaneous exploratory behavior (Open Field Testing) and load-bearing asymmetry (Incapacitance Testing) was conducted to measure pain associated behaviours. End point blood glucose and liver weight were measured. Rats were compared through measures of OA progression including Safranin-O staining with OARSI scoring, immunohistochemistry for cartilage matrix breakdown products, and picrosirius red staining for collagen structure.

Our second cohort of rats (n=6/group), 300-350g male Sprague-Dawley rats were subjected to sham control surgery or ACLT/PMMx surgery and systemically treated with GSK0660, GSK3787, vehicle control DMSO, or saline control for six days per week, starting one day post-surgery, for two or four weeks (early, and moderate OA). End-point weight, blood glucose and liver weight were measured for all animals. Gait and MicroCT analysis were performed in animals 4 weeks post surgery, while classical OA histopathology was scored for subchondral bone and cartilage 2, and 4 weeks post-surgery.

Results: Rats treated with either PPARdelta inhibitor were not different in physiological profiles (blood glucose, liver weight, body weight) from vehicle treated or SHAM operated animals in either cohort, at 2 or 4 weeks post-surgery. In our first cohort, vehicle treated rats demonstrate changes in behaviour after ACLT/PMMx surgery, such as decreased vertical activity and increased rest time. In contrast, ACLT/PMMx animals treated with PPARdelta inhibitors or SHAM operated animals do not demonstrate these changes in behaviour post-surgery. However, preliminary OARSI scoring indicate minimal differences in subchondral bone and cartilage damage between inhibitor treated and vehicle treated rats post-surgery.

In our second cohort, 2 weeks post-surgery our preliminary results indicate very early OA development in all groups except sham animals in the medial and lateral compartment cartilage, with changes such as cell death and proteoglycan loss. Subchondral bone changes, such as increased basophilia, and marrow changes are evident in these groups as well. 4 weeks post-surgery, DMSO-vehicle controls, saline-treated controls, and GSK0660 treated animals present with greater cartilage damage to the medial tibial plateau compared to sham operated animals, while GSK3787 treated animals do not. Similarly, DMSO-vehicle treated controls, saline-treated controls and GSK0660 treated animals present with greater damage to the medial femoral condyle compared to sham treated animals, unlike GSK3787 treated animals.

Conclusions: This study suggests that PPARdelta inhibition may have a role in chondroprotection at intermediate stages of OA progression. Similarly, PPARdelta may have a role in modulating pain-like behaviour in OA. However, additional studies are required, in particular with regard to the differential effects of the two PPARdelta inhibitors at the 4-week time point.

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OSTEOCHONDRAL PATHOLOGY IN MURINE MODELS OF ANTERIOR CRUCIATE LIGAMENT INJURY: IMPLICATIONS FOR THE PATHOGENESIS OF OSTEOARTHRITIS

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Purpose: Post-traumatic osteoarthritis affects approximately 50% of patients with an anterior cruciate ligament (ACL) tear. The magnitude of joint trauma and destabilisation likely play a role in the risk of OA onset and progression however, the relative effects of low versus high load ACL injuries have not been extensively explored. The aim of this study was to examine OA-related structural and molecular changes in ACL injured joints from two unique murine models of injury: surgical transection (ACLT, no injury load) versus mechanical rupture (ACLR, compressive joint overload).

Methods: ACL injury was induced in the right hindlimb of 10-week-old, male C57BL/6 mice by either surgical transection (ACLT) or mechanical compression (ACLR). Separate un-injured mice and sham-surgery controls were included for comparison. At 7, 14, 28 and 56 days post-injury, joints were harvested for histology (n=7) or gene expression using the isolated compartments of the medial and lateral tibial plateau (n=5). The medial and lateral tibial plateaux were evaluated separately for both outcome measures. OA severity was assessed histologically using semi-quantitative scores for: proteoglycan loss, cartilage structural damage and subchondral bone remodelling. Quantitative PCR was performed on the medial and lateral tibial plateaux to investigate key molecules implicated in osteochondral pathology in OA.

Results: OA pathology was observed in both the medial and lateral compartments of ACL-injured knees but was typically worse on the medial side of the joint. Tibial cartilage pathology (proteoglycan loss and cartilage structural damage) was more rapid and severe following ACLR compared with ACLT. While subchondral bone remodelling was evident in all groups (especially medially), osteocyte cell loss was only evident after ACL injury, and osteochondral damage was only found in ACLR joints. The spatiotemporal progression of structural OA was paralleled by the upregulation of genes associated with both cartilage and bone pathology: *ColX, Adamts4, Mmp2, Mmp13, Timp1, Timp3, Col1, Dmp1, Opg, Hhip and Ptch.* Increased expression of *Col2* and *Adamts5* was only seen after ACLR.

Conclusions: ACL-injury induced by mechanical compression (ACLR) resulted in a more rapid and severe degeneration of the knee joint compared to surgically induced ACL tears (ACLT). The differential changes in gene expression between the two models provides further evidence that the initial mechanism of injury (i.e. low versus high load trauma) can significantly alter the biological response of the affected joint tissues. These specific molecular changes and their timing may