ACL repair (BE-repair) and bio-enhanced ACL reconstruction (BE-ACLR) is improved when compared to traditional ACL reconstruction (ACLR) or ACL transection with no treatment (ACLT).

Methods: With IACUC approval, 31 adolescent minipigs underwent surgical ACL transection in one knee followed by BE-repair (n=8), BE-ACLR (n=8), ACLR (n=8), and no treatment (ACLT; n=7). After 12 months of healing, the articulating surfaces of the surgically treated and contralateral ACL intact knees were macroscopically graded following application of India ink using a five point scale (0=no changes; 1=intact surface with color changes; 2=surface fibrillation; 3=exposed bone10%). The surface areas of all lesions were determined using calipers and an elliptical fit. A mixed linear model was used to make comparisons between treatments (BE-repair, BE-ACLR, ACLR, and ACLT) and compartments (medial femoral condyle, lateral femoral condyle, medial tibial plateau, and lateral tibial plateau). Similar analyses were performed to compare the lesion areas within each compartment. All statistical analyses were done on the difference between the surgical and contralateral ACL-intact knee within each animal.

Results: We found significant mean differences in cartilage scores between treatments ($p=0.05$) and compartments ($p<0.01$). The mean difference ± confidence interval for BE-repair, BE-ACLR, ACLR and ACLT (pooled across compartments) were 0.24±0.193, 0.16±0.241, 0.48±0.181, and 0.66±0.392, respectively. Only the knees treated with BE-ACLR did not have increased chondral injury on the surgical side. For the lesion area measurements, the treatment effect was statistically significant in the medial femoral condyle ($p=0.012$). The mean difference ± confidence interval between the surgical and contralateral ACL-intact knees for BE-repair, BE-ACLR, ACLR and ACLT were 5±17.8mm2, -19±23.1mm2, 40±31.9mm2, and 57±51.5mm2, respectively. Both the BE-repair and BE-ACLR procedures resulted in mean differences between the operative and non-operative side that were not significantly different from zero. It should also be noted that there were no lesions in either the surgical or contralateral ACL intact knee in the lateral femoral condyle or medial tibial plateau for any animal undergoing BE-repair.

Conclusions: ACL transection and ACL reconstruction both resulted in increased chondral damage of the knee at one year after surgery as noted in humans. In contrast, treatment of the ACL transection with either bio-enhanced repair with CPC or ACL reconstruction augmented with CPC prevented this increased chondral damage. These data suggest that the intra-articular application of CPC may be chondroprotective.

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CHRONICITY PRODUCES DIFFERENTIAL ALTERATIONS IN PAIN BEHAVIORS IN MURINE OSTEOARTHRITIS

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Purpose: Osteoarthritis pain is a growing problem due to an expanding elderly population and lack of safe and effective therapies. Understanding the origins and pathogenesis of chronic arthritis pain is important for developing effective therapies. Osteoarthritis pain does not correlate well with radiographic severity, and loss of function in patients with osteoarthritis may result from pain, from altered biomechanics or from toxicity of analgesics. In order to better understand the relationship between osteoarthritis, pain, and function, we measured different types of pain behaviors in mice with osteoarthritis as a function of age, duration of arthritis and in response to different analgesic treatments.

Methods: Collagenase (10 IU) in 10 μl was given by intra-articular (IA) injection into the left knee of 4-week-old C57Bl6 male mice. This produced osteoarthritis that was identifiable histologically after 4 weeks. Arthritic mice were compared to uninjected naïve mice of the same age at 4 weeks and 6 weeks after collagenase injection and to arthritic mice treated with IA analgesics. Analgesics tested were IA morphine sulfate (0.7 mg/kg in 5 μl) or IA Botulinum toxin type A (BoNT/A) (0.02 IU given 3 d before testing). Pain behavior measures included evoked pain response to repetitive firm palpation of the knee for 1 minute, voluntary, spontaneous nocturnal wheel-running, mechanical withdrawal thresholds by Von Frey filament testing and digitized video gait analysis using DigiGait™ (Mouse Specifics, Inc, Quincy, MA). The nonarthritic knee was the internal nonpainful control.

Results: At both 4 and 6 weeks after collagenase injection, evoked pain responses in arthritic knees were increased, but this response was 65% greater at 4 weeks than at 6 weeks. Arthritis caused an increased swing/stride ratio measured by gait analysis and increased mechanical allodynia in the arthritic limb by Von Frey filament testing at both 4 and 6 weeks. Von Frey testing in the normal right limb revealed allodynia at 4 weeks but an increased pain threshold at 6 weeks. Spontaneous nocturnal wheel-running was reduced in both the 4 and 6 week arthritic groups, as well as in 6 week naïve mice. Both BoNT/A and Morphine were effective analgesics at 4 and 6 weeks as measured by evoked pain but did not normalize gait function at either time point. Only morphine normalized the threshold for mechanical allodynia to Von Frey filament testing at 4 weeks, but IA BoNT/A was more effective at 6 weeks.

Conclusions: IA injection of collagenase in mouse knees produces arthritis pain that can be measured by various methods at 4 weeks and persists to 6 weeks. Both opioids and BoNT/A given IA are effective analgesics when pain is measured by evoked pain behavior. Changes in functional measures such as gait analysis do reflect the development of arthritis pain but are not clearly normalized by analgesia and may be due not only to pain but to biomechanical changes in the joints. Chronic osteoarthritis pain produces mechanical allodynia, one indication of peripheral sensitization. Mechanical allodynia may be decreased by opioids if arthritis pain is not longstanding but IA BoNT/A may be more effective for sensitization due to arthritis that is more chronic. More work needs to be done to determine which pain behaviors best measure chronic pain in murine arthritis and are sensitive for detecting analgesia in order to use preclinical models for testing potential new analgesics.

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LDL RECEPTOR DEFICIENCY RESULTS IN INCREASED OSTEOPHYTE FORMATION DURING EXPERIMENTAL OSTEOARTHRITIS BOTH UNDER LOW AND HIGH CHOLESTEROL CONDITIONS

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Purpose: Synovial macrophages have previously shown to be involved in joint destruction during experimental collagenase-induced osteoarthritis (OA). The low density lipoprotein (LDL) receptor expressed by synovial macrophages is involved in transport of cholesterol, carrying lipoprotein particles into cells, thereby regulating cholesterol homeostasis. In the present study we investigated whether the LDL receptor is involved in joint destruction during experimental osteoarthritis both under normal and high cholesterol conditions.

Material and Methods: LDL receptor deficient ($LDLR^{-/-}$) mice and their wild type (WT) controls received either a high cholesterol or control diet for 120 days. Experimental osteoarthritis was induced by injection of collagenase into the mice's knee joints on day 84 and 86. Paraffin sections of total knee joints were stained with safranin-o or haematoxylin-eosin to determine OA development. Synovial activation (thickening of the lining layer) was measured using an arbitrary scale from 0 to 3. Cartilage destruction was determined in four cartilage surfaces (lateral and medial femur and tibia) using the OA score (with a maximum of 30 per knee joint) developed by Pritzker *et al* (2006) and adapted by our lab for mice. Size of osteophyte formation was measured on the edges of the femur/tibia area using image analysis. Results are depicted as mean ± SD.

Results: On day 36 after induction of collagenase-induced OA, WT mice which received a normal diet (n=10) developed moderate synovial activation (1.4 ± 0.6), cartilage destruction (6.1 ± 2.6) and osteophyte formation (32.4 μm^2 ± 25.4). In $LDLR^{-/-}$ mice (n=10) no significant differences were found on synovial activation or cartilage destruction when compared to WT controls. In contrast, mean osteophyte formation was tremendously increased by 345 % suggesting that the absence of the LDL receptor induces osteophyte formation in the OA knee joint.

On day 36 after OA induction, the bodyweight of WT mice receiving a high cholesterol diet (n=10) had increased by 21 % compared to WT mice which received a normal diet. Although synovial activation and cartilage destruction was not altered, osteophyte formation was over 33 times higher ($p = 0.0495$) in the medial femur of the high cholesterol group. $LDLR^{-/-}$ mice which received a high cholesterol diet (n=10) expressed high cholesterol levels (500 % higher when compared to WT) within the serum and a significantly increased thickening of the lining layer consisting of macrophages containing high amounts of fat as seen after staining of total knee joints with red oil. Although the OA knee joints of WT mice already showed increased osteophyte formation, the OA knee joints of $LDLR^{-/-}$ developed even higher osteophyte formation (2.7 times higher in the in the lateral tibia; $p = 0.0063$) indicating that the absence of the LDL receptor also induces osteophyte formation under high cholesterol conditions.

Conclusions: LDL receptor deficiency induces osteophyte formation during collagenase-induced osteoarthritis both under low and high cholesterol conditions.

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JOINT HEMORRHAGE ACCELERATED JOINT CONTRACTURE IN IMMOBILIZED KNEE IN RATS

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Purpose: Joint immobilization is commonly used as a treatment for joint injuries and diseases. However, it also causes unfavorable outcomes such as joint contracture. In our previous reports, joint immobilization caused adhesion and shortening of the joint capsule and restricted range of motion. Joint hemorrhage occurs by intra-articular fractures, ligament ruptures and haemophilia. Some researchers have reported blood-induced joint damage using animal models. Although influence of blood on a synovial membrane (SM) and cartilage matrix was reported, the precise mechanism is still controversial. The purpose of this study was to elucidate capsular changes after single blood injection in immobilized knees in rats.
Methods: **Animals:** The unilateral knee joints of Sprague-Dawley rats aged 12-week old were immobilized at 150° of flexion with a plastic plate and metal screws for various periods (1, 3 days, 1, 2, 4, and 8 weeks). Sham operated rats had holes drilled in the femur and tibia with screws, but the plate was not inserted. After the operation, the rats were divided into three groups: Immobilized-blood injection (Im-B) group, Immobilized-normal saline injection (Im-NS) group, and Sham-blood injection (Sm-B) group. Fifty μl of autologous blood were administered intra-articularly for the Im-B and Sm-B groups just after the surgery. The same amount of normal saline was administered for the Im-NS group.
Histology & Immunohistochemistry (IHC): Paraffin embedded 5-μm thick sagittal sections in the medial midcondylar region of the knee were made. The sections were stained with Elastica-Masson to observe morphological changes of the SM and the capsule, and with Perls' Prussian blue to visualize iron deposition in the SM and capsule. The expression patterns of CD68, TGFβ1, and collagen types I and III in the capsule were evaluated by IHC.
Scanning acoustic microscope (SAM): In general, sound speed is in proportion to the square route of Young's elastic modulus. SAM can measure sound speed of tissues on slide glass in situ. We set the region of interests and their average sound speed was calculated with a gray scale SAM images with image analysis software.
Results: Histology & IHC: Absorption of the injected blood was delayed and made severe adhesions in the Im-B group (Fig. 1A-C). Shortening of SM was observed in the capsules due to adhesion of the opposing capsule, the articular cartilage, and the meniscus. The length of posterior SM in the Im-B group was significantly shorter than that of the other groups from 1 to 4 weeks (Fig.1D and E). The iron deposition in the capsule was observed in the Im-B and Sm-B groups (Fig. 2A-C). Strong immunoreactivity of CD68 and TGF-β1 were observed in the adhesion areas in the Im-B group (Fig.2D- I). However, the staining intensity of collagen types I and III did not change in the Im-B group compared to the other groups.

SAM: The low sound speed areas decreased and high sound speed areas increased in the posterior capsule in the immobilized group (Im-B and Im-NS) (Fig.3A- C). The sound speed of posterior capsule in the Im-B group was significantly higher than that in the Sm-B group (Fig. 3D and E).
Conclusions: These data indicated that joint immobilization and blood injections caused irreversible capsular changes. Joint hemorrhage is a risk factor for joint contracture, and drainage of the blood or short immobilization periods might be a good strategy to avoid joint contracture.

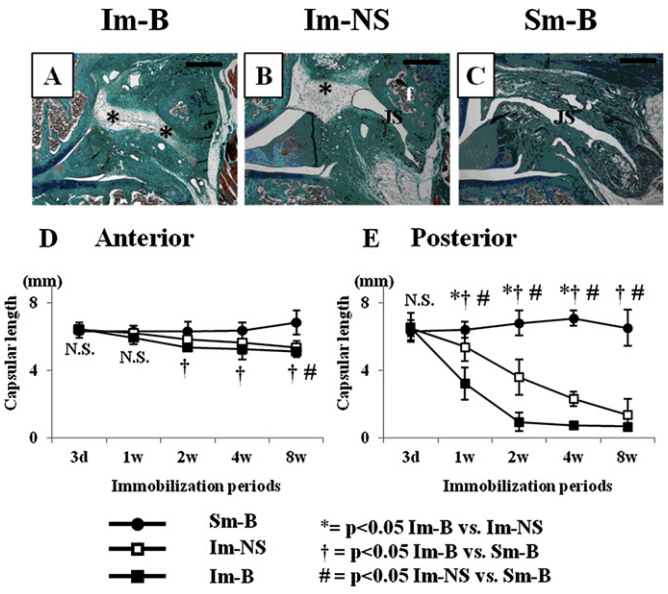

Figure 1. A-C: Posterior Capsule at 2 week. Scale bars = 500 μm.
JS: Joint space, *Arrowhead*: Positive cells, *Asterisks*: Adhesion area.
D and E: Length of the synovial membrane (SM).
D: Total Anterior length, E: Total posterior length.

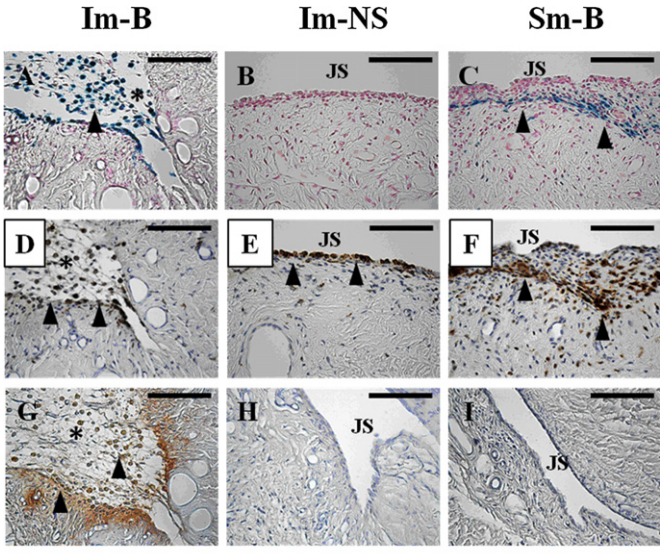

Figure 2. Posterior Capsule at 1 week. Scale bars = 100 μm.
A-C: Perls' Prussian blue, D-F: CD68, G-I: TGF-β1
JS: Joint space, *Arrowhead*: Positive cells, *Asterisks*: Adhesion area.