were observed in the adhesion area in the Im-B group, they were not in the Im-NS group.
Joint angle: In the Im-B and Im-NS groups, the joint angle was significantly reduced compared with that in the Sm-B group after 2-week immobilization. The joint angle of the Im-B group was significantly smaller than that of the Im-NS group at 2 and 8 weeks (Fig. 2).
Conclusions: We revealed that absorption of the injected blood was delayed and made severe adhesions in the Im-B group, which might contribute to restriction in ROM. Intra-articular hemorrhage is a risk factor of joint contracture, and drainage of the blood or short immobilization periods might be a good strategy to avoid joint contracture.

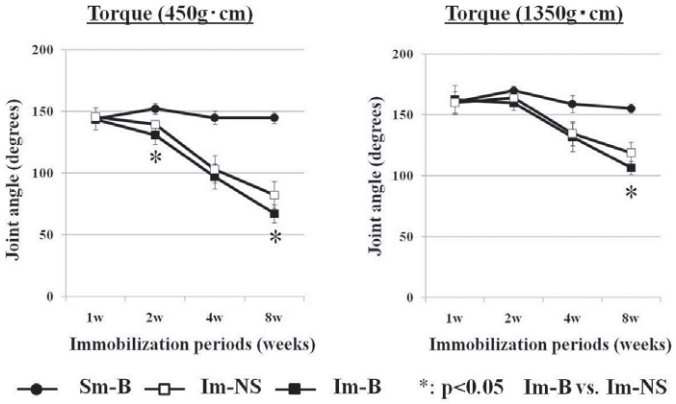

Fig. 2.

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IN A CANINE IN VIVO MODEL, ARTIFICIALLY ENHANCED NONENZYMATIC GLYCATION OF CARTILAGE DOES NOT LEAD TO DEVELOPMENT OF OSTEOARTHRITIS UPON ENFORCED JOINT LOADING

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Background: Osteoarthritis is a highly prevalent disease, age and loading being the main risk factor. The age related accumulation of advanced glycation endproducts (AGEs) adversely affects the mechanical and biochemical properties of cartilage. Animal models can be used to study these effects.
Purpose: The hypothesis that accumulation of cartilage AGEs in combination with enhanced loading induces osteoarthritis and that loading alone will be insufficient to induce OA was tested in an *in vivo* canine model.
Methods: To artificially increase cartilage AGEs, right joints of 8 dogs were repeatedly injected with ribose/threose in PBS, left joints with PBS alone as a control. The dogs were exercised actively in outdoor pens to enhance loading. After 54 weeks joint tissues of all dogs were analyzed for biochemical and histological features of OA.
Results: Cartilage pentosidine (a marker of AGE) levels were 20 fold enhanced ($p = 0.001$ *vs.* PBS injected joints) at the end of the experiment. On average the macroscopic cartilage damage was slightly more severe in the AGEd compared to the PBS injected joints (0.19 ± 0.07 vs 0.33 ± 0.12 respectively; $p = 0.084$). On average the proteoglycan (PG) synthesis is lower in the AGEd joints compared to the PBS injected joints (2.35 ± 0.4 vs 1.91 ± 0.23; $p = 0.05$). For the % total and newly formed PG (42.14 ± 4.5 vs 39.71 ± 4.09 and 18.52 ± 3.04 vs 16.85 ± 2.54, respectively), as well as the PG content (32.35 ± 1.16 vs 32.71 ± 2.75) no statistically difference could be found between both groups.
Conclusions: Artificial AGEing of a joint, leading to cartilage mimicking a pentosidine level of very old animals, is insufficient to develop OA in case of prolonged active loading.

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HYPOXIC CONDITIONS IN THE CAPSULE AFTER JOINT IMMOBILIZATION

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Introduction: Joint immobilization is a useful and commonly performed treatment modality in orthopaedics. However, it also causes unfavorable outcomes such as joint contracture, periarticular osteoporosis, and cartilage degeneration. Once joint contracture is established, it is extremely difficult to regain a full range of motion (ROM) with vigorous and extensive rehabilitation, or even with surgical treatment. In our previous reports, ROM increased after the posterior capsular release in a rat knee flexion contracture model, which indicated the capsule was one of the main causes of joint contracture. Further, angiogenesis factors of transforming growth factor-β1 and connective tissue growth factor increased in the capsule after prolonged immobilization. This result might indicate presence of hypoxia in the capsule after immobilization.
The purpose of this study was to elucidate the changes in the number of blood vessels and cells in the capsule, and presence of hypoxia by hypoxyprobe-1 (HP-1) stain in the rat knee contracture model.
Materials and Methods: *Animals*: Unilateral knee joints of adult male Sprague-Dawley rats (body weight 380–400g) were immobilized at 150° of flexion with a plastic plate and metal screws for various periods (3 days, 1, 2, 4, 8 and 16 weeks) (immobilized group, $n = 5$/each period). Sham operated rats had only screws inserted unilaterally for the same experimental periods (control group, $n = 5$/each period). The other 5 rats were prepared for HP-1 injection by the same immobilization methods (1, 4, and 8 weeks) and the contra-lateral knee was used as a control.
Tissue Preparation: Paraffin embedded 5-μm thick sagittal sections in the medial midcondylar region of the knee were made. The sections were immunostained with a rabbit polyclonal alpha smooth muscle actin (α-SMA) antibody (abcam, dilution 1:100) to count the number of blood vessels. The sections were stained with Elastica-Masson(E-M) to count the number of cells in the capsule. The sections after HP-1 injection were stained with hypoxyprobe-1 (hypoxyprobe inc., dilution 1:400).
Number of Blood Vessels and Cells: The capsule was divided into 4 areas (antero-superior, antero-inferior, postero-superior and postero-inferior subdivisions), and the number of blood vessels and the cells in the capsule per unit were counted using image analysis software.
Statistics: Differences between the immobilized group and the control group were compared at each time point by unpaired t-test. Data were expressed as mean ± SD. A value of $p < 0.05$ was accepted as statistically significant.
Results: *Number of Blood Vessels*: The number of blood vessels in the antero-inferior and postero-inferior capsule per unit significantly decreased in the immobilized group compared with the control group after 4 weeks (Fig. 1).
Number of Cells: The number of cells per unit in the control group did not differ throughout the experimental periods both in the anterior and the posterior capsule. In immobilization group, the number of cells per unit in the capsule peaked at 3 days and gradually decreased after 1 week. The number of cells per unit in the immobilized group was significantly higher than that in the control at 3 days, 1 week and 2 weeks and significantly lower at 8 and 16 weeks (Fig. 2).
Immunohistochemistry of HP-1: HP-1 was detected in the capsule around the blood vessels, and the intensity in the posterior capsule was much stronger in the immobilized group compared to the control group throughout the experimental periods (Fig. 3).

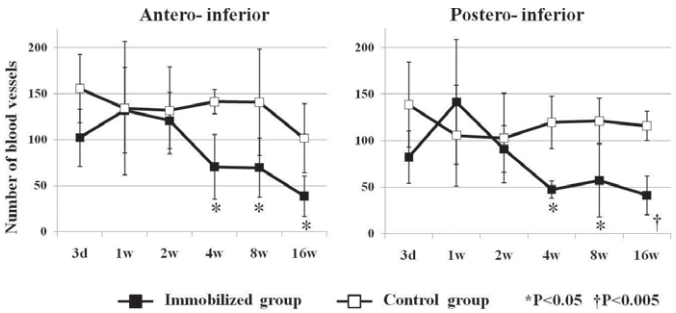

Fig. 1.

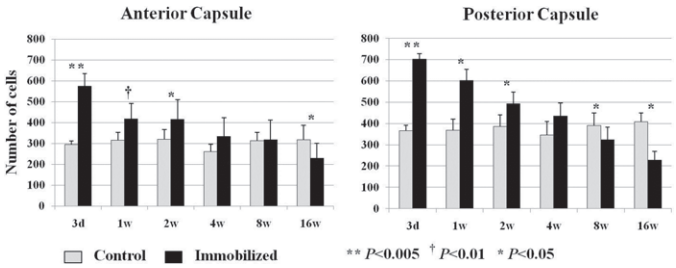

Fig. 2.

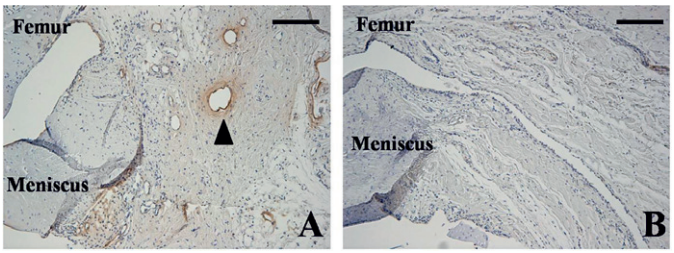

Fig. 3. Arrowhead: strong staining. Scale bar: 200 μm.

Conclusions: We revealed in this study that joint immobilization induced hypoxic condition in the capsule. The decreased number of blood vessels and the increased number of cells might indicate decreased blood flow and fibrosis in the capsule. Hypoxia is an important factor of deterioration of joint contracture after immobilization.

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A NEW IMPACTION SYSTEM TO CREATE "CRITICAL" CARTILAGE INJURY IN LIVING RABBIT KNEES

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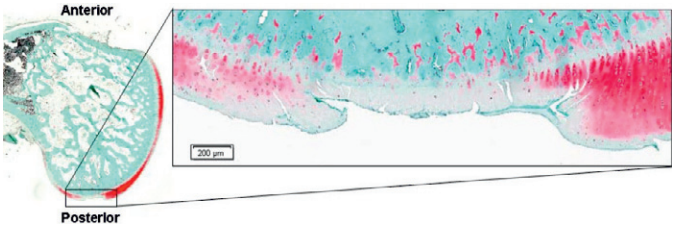
Purpose: In post-traumatic OA, death and dysfunction of chondrocytes associated with acute cartilage injury presumably plays an important role in triggering the pathomechanical cascade that eventually leads to whole-joint degeneration. To study details of the disease mechanisms, or to pilot treatment to amend the disease process, a survival animal model in which OA predictably develops after mechanically-introduced acute cartilage injury is crucial. A novel impaction system has been developed to create such acute cartilage injury in living rabbit knees. The present study aimed to test if this system would be capable of creating cartilage injury on the medial femoral condyle in the primary weight-bearing region (where cartilage is thickest), with a level of injury severity regarded as "critical" in the sense of the cartilage injury created causing progressive cartilage degeneration.

Methods: With institutional approval, twelve New Zealand White rabbits received blunt impaction insult to the left knee. By approaching through a posterior arthrotomy, the posterior aspect of the medial femoral condyle was bluntly impacted using a custom drop-tower device. In this system, the rabbit was positioned prone, with the left thigh mounted on the leg holder. The distal femur was placed in a V-shaped groove, and was secured using a cannulated bone pusher, with guidance of a 1.25 mm K-wire embeded into the posterior femoral metaphysial cortex. A metal platen with a flat impaction face (5 mm diameter) was then placed on the medial femoral surface, and an impaction force was delivered by a 1.55 kg drop mass. The magnitude of energy delivery was controllable (up to 5.0 joules) by adjusting the drop height. The animals were impacted at 2.0, 3.0, or 4.0 joules, and were sacrificed 1 or 8 weeks post-impaction (n = 2 for each combination of impaction magnitude and test period). The experimental joints were subjected to histo-morphological evaluation of the femoral and tibial surfaces in both medial and lateral compartments. For evaluation of the medial femoral surface, histological sections were prepared at 0.5 mm intervals, and a section that included the most severe cartilage injury was identified.

Results: All of the six experimental joints harvested at 1 week post-impaction had full-thickness cartilage injury on the medial femoral condyle, at a region where cartilage was thickest (Figure). There was no recognizable relationship between injury severity and energy delivery magnitude. However, in the joints harvested at 8 weeks, the most severe injury (cartilage defect reaching the calciified zone) was identified only in the 4J-impaction joints. Findings in the rest of 8-week joints (2J- or 3J-impaction) were variable across specimens, from nearly no damage to full-thickness injury. On the medial tibial surface, all 8-week joints had surface roughness, with cracks reaching the transitional to radial zone.



Conclusions: Although not fully reproducible, critical-level acute cartilage injury was inducible in the primary weight-bearing region of the medial femoral joint surface. Secondary effect on the opposing tibial surface was also suggested. This new impaction system appears to permit modeling OA development following acute cartilage injury in living rabbit knees.

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MEDIAL MENISCUS DESTABILIZATION FOR MODELING CUMULATIVE ABNORMAL CONTACT STRESS IN LIVING RABBIT KNEES

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Purpose: In post-traumatic OA, cumulative abnormal contact stress presumably plays an important role in the disease progression in the chronic phase. To study details of the disease mechanisms, or to pilot treatment(s) to alter the disease process, a survival animal model in which OA predictably develops purely due to cumulative abnormal contact stress is crucial. A novel surgical insult technique has been developed to model localized contact stress elevation in the rabbit knee, with minimal effects on the whole-joint mechanics. The present study aimed to test if this surgical insult would predictably cause cartilage degeneration in living rabbit knees.

Methods: With institutional approval, five New Zealand White rabbits were subjected to medial meniscus destabilization (MMD) surgery. Approaching through a posterior arthrotomy, the posterior horn of the medial meniscus was sharply released from the tibial attachment. Major ligamentous and muscular structures surrounding the knee, particularly the quadriceps tendons, were left uninjured. Eight weeks later, the animals were sacrificed, and the A-P joint laxity was measured for both knees. Then, the experimental joints were prepared for histo-morphological evaluation. Femoral and tibial surfaces in both medial and lateral compartments (at the primary load bearing region for each surface) were rated individually using Mankin score (0–14 points). These results were compared with data archived from previous studies, in which rabbit knee cartilage histology was evaluated 8 weeks after total medial meniscectomy (MMtomy, n = 5), complete anterior cruciate ligament transection (ACLT, n = 10) or sham control surgery (n = 10).

Results: Difference in A-P laxity (neutral-zone length) between the experimental and contralateral knees was minimal (0.21 mm or less). In gross anatomical observation, all (5/5) MMD knees had the medial meniscus still released at the posterior attachment, with the body of meniscus moderately degenerated. Histologically, 4/5 of the MMD knees had distinct cartilage degeneration (Mankin score ≥ 4 points) in the medial compartment, on both the femoral and tibial surface (Figure). All MMD knees had a medial tibial score higher than the 75th-percentile value of the previous sham surgery data, and these scores were comparable with those of the previous ACLT and MMtomy data. The medial femoral score for 4/5 of the MMD knees was higher than the 75th-percentile values of the sham surgery dataset. These elevated scores were higher than most of those in the ACLT dataset, and comparable with those in the MMtomy dataset. Histological changes in the lateral compartment were minimal (≤ 2 points) in most cases.

Conclusions: MMD is a well-accepted surgical insult technique to model OA in the mouse knee. It has been experimentally evidenced that detachment of the posterior attachment of the medial meniscus from the tibia causes significant increase of contact stresses in the medial compartment in the human knee. Our technique allows MMD of the