remained unclear whether synovitis is also related to cartilage alterations or other features in patients with end-stage knee OA, where articular cartilage is almost disappeared. In addition, magnetic resonance imaging (MRI) measurements are sensitive to detect of OA-related structural changes. The aim of this study was to investigate the MRI-detected OA-structural changes of the knee joint those were associated with histological synovitis in patients with end-stage knee OA who underwent TKA.

Methods: The forty patients (female: 88%, 71.1y on average) who fulfilled the American College of Rheumatology criteria for knee OA and required TKA due to the end-stage knee OA of Kellgren and Lawrence grade 4 were enrolled in this study. All participants were performed with 3.0-Tesla MRI. The OA morphological changes, such as (1) cartilage morphology, (2) bone marrow lesions (BMLs), (3) bone cysts, (4) bone attrition, (5) meniscal pathology, (6) osteophyte, (7) synovitis and (8) ligaments were scored using the whole-organ MRI scoring (WORMS) method. The synovial samples were obtained from five regions of interest of the knee joint at the operation. The sections were stained with hematoxylin and eosin. The histological synovitis scores (HSS) were measured by the methods previously described. Next, the sections were stained with TGF-β, COX-2, IL-1β and IL-6. A semi-quantitative analysis of the immune-histochemically stained sections was conducted. Associations between the HSS and WORMS were examined using Spearman's correlation coefficient. The means of each HSS were compared using the Mann-Whitney U test.

Results: Among the eight OA-related morphological changes, the total BML score were significantly associated with the total HSS ($r = 0.64$, $p = 0.02$). BMLs were present in all patients in medial femoro-tibial joint (MFTJ), and were present in lateral femoro-tibial joint (LFTJ) in approximately half (53%) of the patients. The HSS in MFTJ and LFTJ were significantly associated with medial and lateral BML scores [$r = 0.35$, $p = 0.03$ (medial), $r = 0.49$, $p < 0.01$ (lateral), respectively]. The total HSS in patients with BML in LFTJ were significantly increased compared to those without BML in LFTJ ($p < 0.001$). The synovial TGF-β expression in the LFTJ in patients with BMLs in the LFTJ was significantly higher than that in patients without BMLs in the LFTJ, while these differences were not observed in COX2, IL-1β or IL-6.

Conclusions: The BMLs, but not other structural changes detected by MRI, were associated with the histological synovitis and synovial TGF-β expression in patients with end-stage knee OA.

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HIGH-FAT DIET FEEDING AFFECTS OSTEOARTHRITIS PROGRESSION IN A SURGICAL MOUSE MODEL WITH ESTABLISHED AND DEVELOPING METABOLIC SYNDROME

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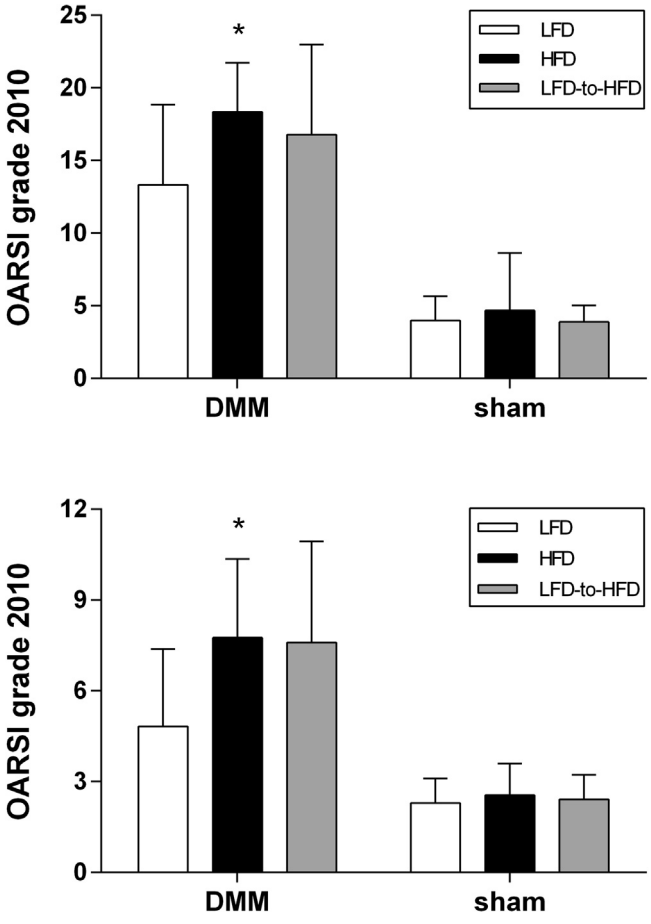
Purpose: Metabolic syndrome (MS) is a major risk factor for osteoarthritis (OA) development. The MS-associated low-grade systemic inflammation may underlie this link. We investigated whether a systemic metabolic burden due to high-fat feeding in mice would aggravate OA progression, in both established as well as developing MS.

Methods: Metabolic syndrome was induced by providing a high-fat diet (HFD, 45% kcal from fat) to 12-week old male C57Bl/6J mice (n=10) for 10 weeks. Control mice of same sex and age received a synthetic low-fat diet (LFD, 10% kcal from fat) during that time period. At 10 weeks the medial meniscotibial ligament (MMTL) of the right knee joint was transected to destabilize the joint and thereby induce OA. The left knee joint served as an internal control and underwent all the surgical procedures except for the MMTL transection (sham). Half of the control mice were switched to HFD at the time of surgery (LFD-to-HFD), to attain a state of developing metabolic syndrome at end-point. Body weights and changes in body composition were monitored during the entire study period. At endpoint, OA severity was graded on Safranin O-stained histological sections of the knee joints according to OARSI recommendations.

Results: Bodyweights of the mice on long- and short-term HFD were significantly different at end-point (HFD, 41.1 ± 6.9 g; LFD-to-HFD, 38.8 ± 3.1 g) compared to mice of the control group (LFD, 31.8 ± 2.1 g). Body composition analysis showed that the increase in body weight almost completely resulted from an increase in body fat mass; lean mass remained constant between groups. Severity of OA was greater in the mice fed a HFD, for both the short-term and the long-term regimen. Separate assessment of the long- and short-term HFD groups showed a statistically significant difference in cartilage degeneration between the HFD and control groups (Figure 1; one-tailed t-test, $p = 0.026$). This effect was mainly observed at the lateral knee compartments, implying that the observed cartilage degeneration at the lateral knee compartments was predominantly diet-induced (Figure 2). Interestingly, analysis of the lateral knee compartments of the LFD-to-HFD group showed a clear trend towards an increase in OA severity compared to control (one-tailed t-test, $p = 0.064$). OA severity did not correlate with body fat mass at end-point.

Conclusions: Our results show that high-fat feeding indeed aggravates OA progression in a surgical mouse model for OA. In this model, the diet-induced OA progression was mainly observed at the lateral knee compartments. No correlation between OA severity and body weight or body fat mass was found, suggesting that the MS-associated inflammation may underlie the diet-induced OA progression.



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CHONDROGENIC PROGENITOR CELLS PROVIDE A NOVEL INSIGHT TO PHAGOCYTOTIC ACTIVITIES WITHIN CARTILAGE

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Purpose: Chondrogenic progenitor cells (CPCs) have been identified on the cartilage surface post injury, as well as in osteoarthritic cartilage. CPCs serve chondroprotective and regenerative functions, but in initial response to cartilage injury, CPCs overexpress markers associated with dendritic cells. Based on these findings we hypothesized that CPCs carry the potential to clear cell and matrix debris through phagocytosis. To test this we compared phagocytotic capacity of CPCs to chondrocytes.

Methods: Scratches on bovine knee cartilage were created to stimulate CPCs migration. After culturing for ten days, CPCs and chondrocytes were isolated and co-cultured with DiO-labeled cell debris for various time periods. Confocal microscopy and flow cytometry analyses were performed to determine phagocytosis events in both cell types.

Results: Confocal microscopy images show CPCs migrating toward acartilage injured (Fig 1A). In CPCs, most DiO-labeled cell debris appeared to be engulfed within the cytoplasm, while in chondrocytes, most of the label appeared to be bound to the cell membrane (Fig 1B). Flow cytometry quantitatively confirmed that DiO+ CPC percentage was significantly higher than chondrocytes at each time point (3hrs, 6hrs, 12hrs and 24hrs) (Fig 2). In addition, DiO+ CPCs increased dramatically (12.58% for 3hrs, 28.63% for 6hrs) and peaked at 12hrs (68.10%), while DiO+ chondrocytes increased slightly (4.18%, 6.66%, 8.08%, and 11.56%, respectively).
Conclusions: CPCs showed much higher and sustained uptake of cell debris than chondrocytes. Though not conclusive, these findings support the hypothesis that CPCs play a role in clearing cell debris in damaged cartilage. The physiologic significance of this activity is unclear, but it is conceivable that it neutralizes the pro-inflammatory activity of cell debris, an essential step in wound healing.

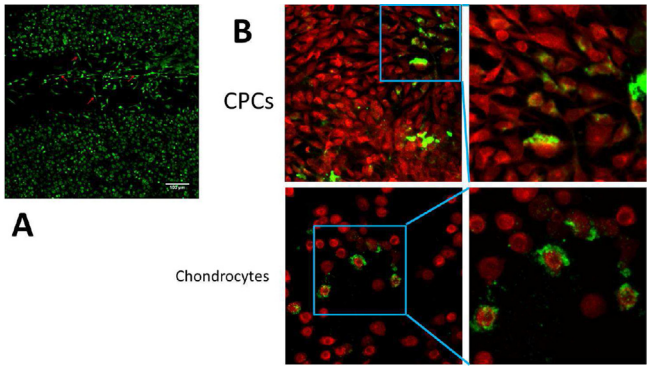
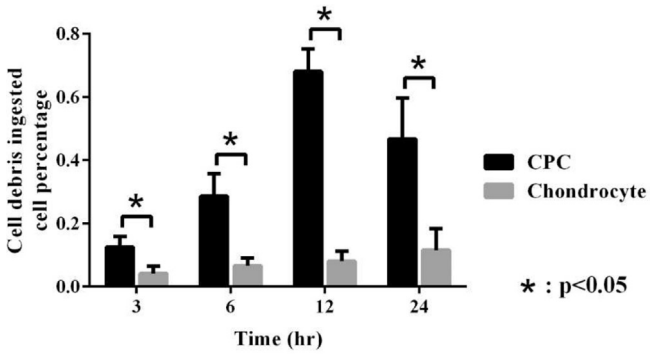


Figure 1. A) Morphological distinction between CPCs and chondrocytes, CPC (long stretched cells indicated by red arrows) residing on the surface of scratch site. CPCs showed dramatic morphological difference compared to the chondrocytes (rounded cells in most upper and lower portion of the figure) B) Visual comparison between CPCs (upper) and chondrocytes (lower) in terms of phagocytosis under microscopy. Cell debris (stained with green-fluorescent DiO label) was vastly ingested into the cytoplasm of CPC. While for chondrocytes, most cell debris was sticking outside the cell membrane.



Flow cytometry quantification assay showed DiO+ cells percentage within CPC population is significantly higher than chondrocytes, along with different rates to phagocytose cell debris for CPCs and chondrocytes (12hr shows highest in CPCs, while gradually increase in chondrocytes).

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SYNOVITIS AND CHANGES IN THE EXPRESSION OF SYNOVIOCYTES MARKERS CD68 AND CD55, DURING THE PROGRESSION OF OSTEOARTHRITIS IN A RAT MODEL

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Purpose: To evaluate synovitis and changes in the expression of the synoviocytes markers CD68 and CD55 during the progression of osteoarthritis (OA) and correlating with the articular cartilage injury in a rat model.
Methods: OA was induced in Wistar rats by unilateral knee menisectomy and post-surgery exercise for 15 min daily during; 1, 5 and 10 days. Data were compared with sham-operated rats in which the capsule and synovial membrane were cut but the meniscus was not removed. Normal rats were used as controls. Joints were fixed with 4% paraformaldehyde in PBS for 12 hr at 4°C and then decalcified in citrate/formic acid solution during 12 days. Joints were embedded in tissue freezing medium and cryosectioned to obtain 6 μm thick slices, which were mounted on gelatin-coated slides. Slices were either stained with Hematoxilin-Eosin (H-E) or processed for inmunofluorescence to detect CD68 and CD55 proteins. H-E stained tissues were observed and captured with an optical microscope while inmunofluorescence stained tissues were observed and captured with confocal microscope (Carl-Zeiss LMS 700). Statistical analyses were performed using the Graph Pad prism 5 program. One-way ANOVA analysis with Kruskal-Wallis multiple comparison test was used to compare means among the experimental groups.
Results: At 1, 5 and 10 day after surgery we did not find differences in the score of synovial inflammation between sham operated and menisectomized joints. Synovitis was characterized by hyperplasia with increased number of lining cell layers. To associate hyperplasia with the amount of synoviocytes, we analyzed the expression of the synoviocytes markers CD68 and CD55. CD68 expression was increased in both sham and OA groups when compared with normal joints. However, we found that synovial membrane of OA groups expressed more CD68 than sham groups. Furthermore, compared to normal group, the expression of CD55 protein decreased similarly in both sham and OA joints at 1, 5 and 10 days. OA hallmarks in articular cartilage were only found in menizectomized joints but not in sham operated joints.
Conclusions: Our results suggest that inflammation of synovial membrane at early stages is not enough to induce OA hallmarks. Therefore, synovitis and the differences in the amount of synoviocytes A and B subtypes might be important factors at the beginning and progression of OA.
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SPECIALIZED PRO-RESOLVING LIPID MEDIATORS IN OSTEOARTHRITIS PATIENTS: EVIDENCE FOR AN ANTI-INFLAMMATORY ROLE

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Purpose: Inflammation is usually a self-resolving process. The resolution phase involves several cells and molecules that act in a coordinated manner to suppress inflammation, help clearance of apoptotic cells and restore tissue homeostasis. Among these molecules, the pro-resolving specialized lipid mediators (SPM) have emerged as biomarkers and potent mediators of the resolution process. We hypothesized that the underlying cause of chronic inflammation in Osteoarthritis (OA) and Rheumatoid Arthritis (RA) could be the failure of activating pro-resolving mechanisms, involving poly-unsaturated fatty acids and their biologically active metabolites.
Methods: To investigate this, we have collected synovial fluid from the knee from 33 OA and 20 RA patients. We measured more than 50 analytes in the cell-free supernatant, including (pro-resolving) lipid mediators, their pathway markers and accompanying poly-unsaturated fatty acids (PUFA), using liquid chromatography combined with mass spectrometry (LC-MS/MS). To this end, we have set-up a novel analytical platform, characterized by a simplified work-up protocol and high-throughput capabilities, making it particularly suitable for clinical studies. Moreover, we have quantified and characterized the cellular infiltrate using flow cytometry and the appropriate combination of antibodies directed against cell surface markers. Additionally, we have measured 11 cytokines in synovial fluids using the multiplex technology.
Results: By using as little as 40 μL of synovial fluid, we could demonstrate the presence of significant amounts of 5-HETE, 12-HETE, 18-HEPE, 15-HETE, 10S,17S-diHDHA, PGE2 and 17-HDHA, a series of other hydroxylated analytes and a number of PUFA in synovial fluid of OA and RA patients. Analyses of lipid mediator concentrations revealed that most lipid mediators had similar levels in OA and RA samples.