in hIL4–10FP group there was no enhanced cartilage degeneration detected compared to the PBS group (fig 3).

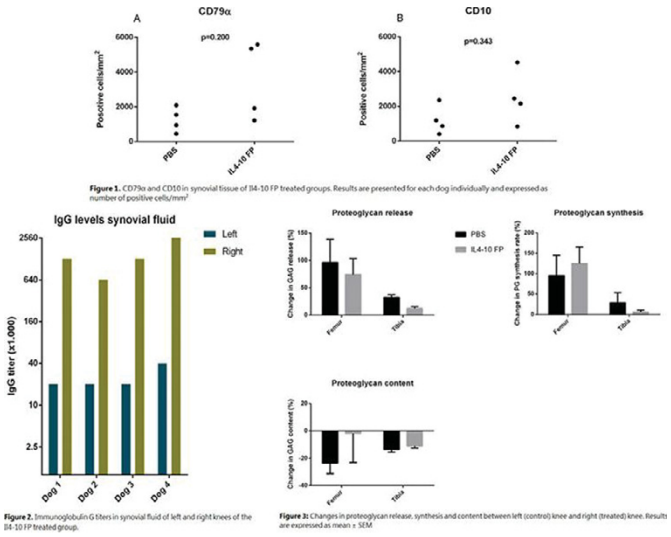

Figure 1. CD79α and CD10 in synovial tissue of Il4-10 FP treated groups. Results are presented for each dog individually and expressed as number of positive cells/mm²

Figure 2. Immunoglobulin G titers in synovial fluid of left and right knees of the Il4-10 FP treated group.

Figure 3: Changes in proteoglycan release, synthesis and content between left (control) knee and right (treated) knee. Results are expressed as mean ± SEM

Conclusions: Repetitive intra-articular injection of human IL4–10FP led to antibody formation in a non-inflammatory canine model of OA. Despite the immune response, proteoglycan turnover parameters were comparable between the two treatment groups, suggesting a beneficial effect of hIL4–10FP. This study also shows that it is not evident to use a human protein in a (canine) animal model, although this is often done. Instead, a species specific protein is warranted. Therefore a canine version of IL4–10FP will be developed to study its DMOAD activity in this model.

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FRI0009 ACCELERATED DEVELOPMENT OF AGING-ASSOCIATED AND INSTABILITY-INDUCED OSTEOARTHRITIS IN 12/15-LIPOXYGENASE DEFICIENT MICE

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Background: 12/15-Lipoxygenase (12/15-LOX) catalyzes the generation of various anti-inflammatory lipid mediators, and has been implicated in several inflammatory and degenerative diseases. However, there is currently no evidence that 12/15-LOX has a role in osteoarthritis (OA).

Objectives: The aim of this study was to investigate the role of 12/15-LOX in the pathogenesis of OA

Methods: The development of aging-associated and destabilization of the medial meniscus (DMM)-induced OA were compared in 12/15-LOX-deficient (12/15-LOX-/-) and wild-type (WT) mice. The extent of cartilage damage was evaluated by histology. The expression of OA markers was evaluated by immunohistochemistry and RT-PCR. Cartilage explants were stimulated with IL-1α in the absence or presence of the 12/15-LOX metabolites, 15-HETE, 13-HODE or LXA4, and the levels of MMP-13, NO and PGE_2 were determined. The effect of LXA4 on the progression of OA was evaluated in WT mice.

Results: The expression of 12/15-LOX in cartilage increased during the progression of DMM-induced OA and with aging in WT mice. Cartilage degeneration was more severe in 12/15-LOX-/- mice compared to WT mice in both models of OA, and this was associated with increased expression of MMP-13, ADAMTS5, iNOS, and mPGES-1. Treatment of cartilage explants with 12/15-LOX metabolites, suppressed IL-1α-induced production of MMP-13, NO and PGE_2, with LXA4 being the most potent. Intra-peritoneal injection of LXA4 reduced the severity of DMM-induced cartilage degradation.

Conclusions: These data demonstrate an important role of 12/15-LOX in OA and suggest that activation of this pathway may provide a novel strategy for prevention and treatment of OA.

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FRI0010 METABOLIC DYSREGULATION ACCELERATES JOINT DEGENERATION UPON MECHANICALLY INDUCED CARTILAGE DAMAGE, DRIVEN BY LOCAL INFLAMMATION; AN IN VIVO RAT STUDY

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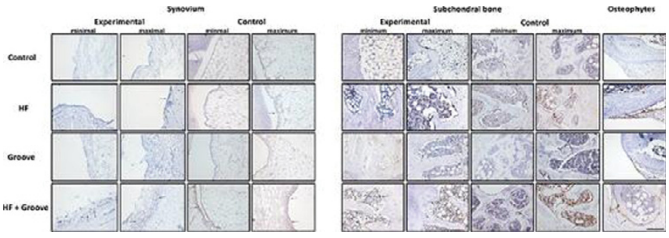
Background: Obesity is a well-known and important risk factor for osteoarthritis (OA). Moreover, obesity is highly associated with the metabolic syndrome (MetS)[1]. Growing evidence indicates that both OA and MetS are low-grade inflammatory conditions with elevation in systemic inflammatory markers. Nonetheless, it is unclear whether MetS low-grade inflammation induces OA, or contributes to the disease.

Objectives: To determine the contribution of metabolic alterations, induced by a High-Fat Diet (HFD), on the onset or progression of OA in a rat model of local cartilage damage.

Methods: Forty Wistar rats (12 weeks old, male), were randomly divided over two groups: twenty rats were fed a HFD (60% of the kcal contained fat:D12492i, Research Diets Inc.) while the other animals received a standard diet. After 12 weeks, local articular cartilage damage was induced on the femoral condyles, in one knee joint according to the groove model in 14 rats of each diet group. Remaining animals served as a control group in each arm. At week 24, serum was collected, subchondral bone was assessed by μCT scan (Quantum FX, PerkinElmar,USA), OA severity was evaluated by rat OARSI histopathology score and macrophage presence with CD68 immunostaining from histological sections was assessed.

Results: HFD feeding resulted in metabolic dysregulation as indicated by significantly increased metabolic parameters (weight, fasting insulin and total cholesterol) compared to the standard fed rats. HFD feeding alone resulted in mild cartilage degeneration (2±1.1 vs 0.58 ±0.7; p=0.06) and synovial membrane inflammation (1.0±0.6 vs 0.3±0.5; p=0.075) both subscores of the rat OARSI histopathology score. However, when HFD feeding is combined with the surgical model of applied local cartilage damage, OA severity is statistically significant increased compared to the local cartilage damage group on a standard diet (6.2±2.1 vs 3.4±1.4; p=0.001). Synovial membrane inflammation (1.3±0.9vs 0.5±0.5; p=0.011) and multiple large osteophyte formation, demonstrated by histology (0.9±1 vs 0.2±0.4; p=0.04) and quantified on μCT (328±349 μm^3 vs 7±14 μm^3; p=0.0001), contributes most to this increased OA severity. Immunohistochemical CD68 expression as observed on both the synovial membrane as well as in the subchondral bone and around the formed osteophytes can explain the increase in selected inflammatory parameters when groove surgery is combined with a HFD (Figure 1).



Conclusions: This study shows that a HFD induces metabolic alterations and increases the inflammatory state of the joint. This by itself does not result in severe OA. However, when adding a HFD to a mild cartilage damage model of OA, joint degeneration is significantly increased. This progression of joint degeneration appears to be driven mainly by inflammatory responses as demonstrated by an increased CD68 expression in both the subchondral bone and synovium membrane with increased osteophytosis. Hence, our findings indicate that systemic metabolic and subsequent inflammatory factors need an additional trigger to contribute to the progression of the OA.

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FRI0011 TARGETING NEUTROPHIL MICROVESICLES TO DAMAGED CARTILAGE USING ANTIBODIES TO POST TRANSLATIONALLY MODIFIED COLLAGEN II

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Background: Microvesicles (MV) are double membrane-bound extracellular vesicles released from the plasma membrane of cells. MV derived from polymorphonuclear neutrophils (PMN) promote tissue protection, and have been demonstrated to penetrate cartilage during inflammatory arthritis and provide protection to the tissue [1].

Collagen type II (CII) is the most abundant protein found in cartilage. We have produced a single chain variable fragment (scFv) antibodies specific to CII modified by reactive oxygen species (ROS), namely anti-ROS-CII_{scFv}. Previously, we have demonstrated the ability of anti-ROS-CII_{scFv} to localise exclusively and deliver payload drugs to the arthritic joint in mice models of rheumatoid arthritis[2].
Objectives: To test our hypothesis that anti-ROS-CII association with MV might i) target delivery of MV to inflamed joint and/or ii) enhance the avidity of the scFv (several scFv can be loaded in each MV) and may thus increase localisation and enhance therapeutic efficacy.
Methods: Cy5.5 labeled Anti-ROS-CII_{scFv} were loaded on fluorescently labelled human PMN MV by aqueous energy dissemination using a sonic dismembrator [3–4]. Anti-ROS-CII_{scFv} MV incorporation was confirmed by Imagestream^X analysis. Anti-ROS-CII_{scFv} MV were tested by ELISA to assess the retention of antibody binding capabilities.
Results: Positive incorporation of Anti-ROS-CII_{scFv} upon MV was observed by flow cytometric analysis. ELISA demonstrated the ability of the anti-ROS-CII loaded MV to bind strongly to ROS-CII following incorporation into MV.
Conclusions: In this study, we have demonstrated a simple, efficient and cost effective way of antibody targeting that retains antibody function. Such technology has the potential to increase efficacy of existing therapies by ensuring specific targeting. Future *in vivo* studies will assess the ability of the Anti-ROS-CII_{scFv} MV to localise to arthritic joints.
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FRI0012 ROLE OF VOLATILE COMPOUNDS RELEASED BY SYNOVIAL FLUID IN THE DIAGNOSIS OF OSTEOARTHRITIS AND RHEUMATOID ARTHRITIS OF THE KNEE JOINT

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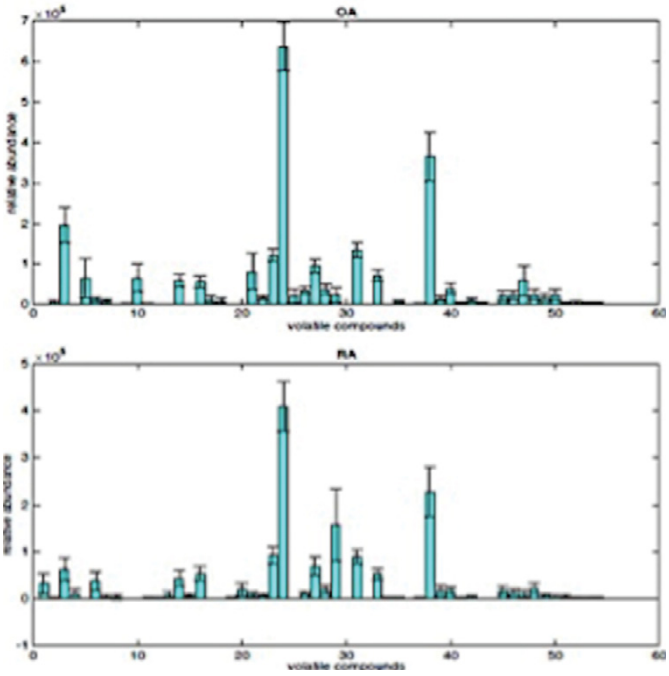
Background: Synovial fluid (SF) receives protein contribution from the tissue around: cartilages, synovial membranes and bones. The presence of inflammation and oxidative stress alters the its chemical composition. In particular, inflammation modulates the release of volatile organic compounds (VOCs) that are the product of reactive oxygen species and free radicals excreted by mithocondria during oxydative stress (1). Articular inflammation plays a major role both in Osteoarthritis (OA) and Rheumatoid arthritis (RA), thus, the identification of specifics VOCs associated with inflammation in the SF may represent a suitable procedure to facilitate a diagnosis and a better characterization of these diseases. E-noses are versatile instruments based on arrays of partially selective gas sensors system that do not provide specific information about the individual molecules but can detect a large spectrum of VOCs to provide a discrimination among samples classified according to their chemical composition (VOC pattern) (2).
Objectives: Aim of this study was to prospectively investigate whether analysis of volatile compounds (VOCs) emitted from knee synovial fluid can identify differences between osteoarthritis (OA) and rheumatoid arthritis (RA).
Methods: VOCs Profile emitted by knee synovial fluid of 10 OA patients was compared with that of 25 RA patients using gas chromatography and mass spectrometry (GC-MS) and a gas sensor array (electronic nose) made by an ensemble of metalloporphyrins coated quartz microbalances. Patients' data are summarized in Table 1. Data were analyzed by principal component analysis (PCA), partial least squares discriminant analysis (PLSDA). Permutation analysis and area under curve (AUROC) of receiver operating characteristics (ROC) curves were used to characterize the classifier performance.
Results: GC-MS analysis identified 55 VOCs in the headspace of synovial fluids. The ANOVA analysis of the relative abundance indicated five VOCs significantly different between OA and RA. The abundance of five compounds allowed to identify OA with respect to RA with an accuracy of 82% (sensitivity: 0.90, specificity: 0.80, AUROC=0.92, 99.7% CI). The signals of the electronic nose sensors allowed to classify the studied subjects in OA or RA. In particular, OA patients could be distinguished from that of RA patients with an accuracy of 100% (sensitivity: 1, specificity: 1, AUROC=1, 99.9% CI).) (Figure 1). However, no single VOC was specific for OA or RA.

Figure 1. Mean and standard deviation of relative abundance of the 54 compounds.



Conclusions: This study shows that OA and RA patients exhibit qualitative and quantitative differences in the chemical compositions of knee synovial fluid. These differences may be attributed to five volatile compounds and can be detected by an electronic nose which may represent a suitable diagnostic tool for diagnosis and characterization of OA vs. RA.
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FRI0013 ACPA-INDUCED MOBILITY OF PRIMED SYNOVIAL FIBROBLASTS: THE MISSING LINK BETWEEN ACPA-INDUCED BONE LOSS AND SYNOVIAL CHANGES

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Background: Anti-citrullinated proteins antibodies (ACPAs) injected in mice induce IL-8 dependent bone loss and arthralgia, but no synovial changes. We hypothesized that additional stimulus, sensitizing the synovial compartment to ACPA effects, is needed for the transition from bone to synovial pathology.
Methods: Synovial fibroblasts (SFs) were isolated from synovial tissue of RA patients by enzymatic digestion. Polyclonal ACPA and other non-ACPA IgGs were separated from peripheral blood of RA patients by affinity purification on a cyclic citrullinated peptide (CCP)-2 column. SF migration capacity was tested by scratch-assays in starved and non-starved cultures treated with ACPAs, with or without presence of IL-8. The results were evaluated by NIH ImageJ software. SF adhesion was analyzed by xCELLigence System Real-Time Cell Analyzer (ACEA bioscience). Peptidylarginine deiminases (PAD) expression and protein citrullination were evaluated by immunohistochemistry. The role of signaling pathways in the ACPA-mediated SF modulation was analyzed by using specific signal inhibitors and by monitoring protein phosphorylations using western blot.
Results: Serum starvation of SF increased citrullinated proteins and PAD expression. Starved but not non-starved SF showed an increased mobility index following polyclonal ACPA stimulation to a mean±SD fold increase of 2.6±0.5. This effect was abolished by PAD inhibition as well as ACPA blocking with citrullinated but not native fibrinogen. Exogenous pro-inflamamatory cytokines (IL-8 and TNF) synergistically increased SF mobility when added together with ACPA. Phosphorylation and inhibition studies of intracellular signalling pathways