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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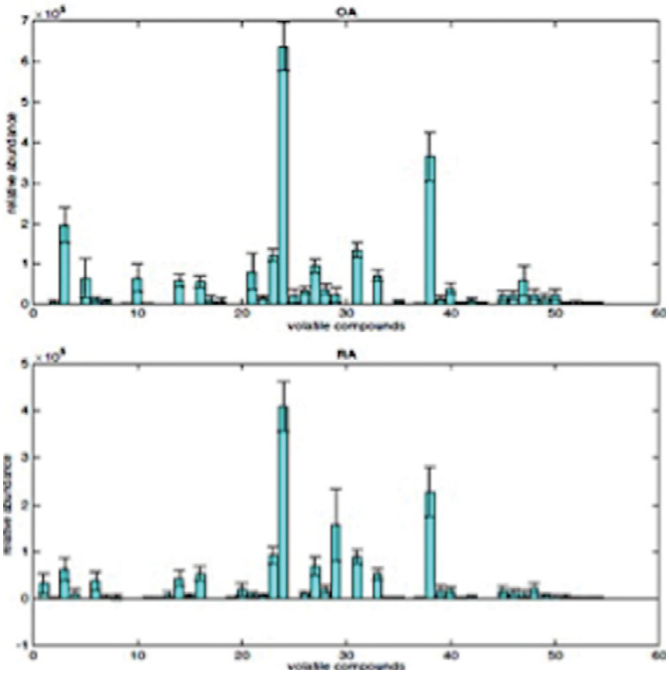
Background: Synovial fluid (SF) receives protein contribution from the tissue around: cartilages, synovial membranes and bones. The presence of inflammation and oxidative stress alters the its chemical composition. In particular, inflammation modulates the release of volatile organic compounds (VOCs) 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