

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 arthritis².

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 dismembrator³⁻⁴. 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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Acknowledgements: The author would like to acknowledge Timothy Harrison and Amit Gupta for their contributions to preliminary results. The author would also like to acknowledge Dr Jesmond Dalli for his guidance on methodology.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4842

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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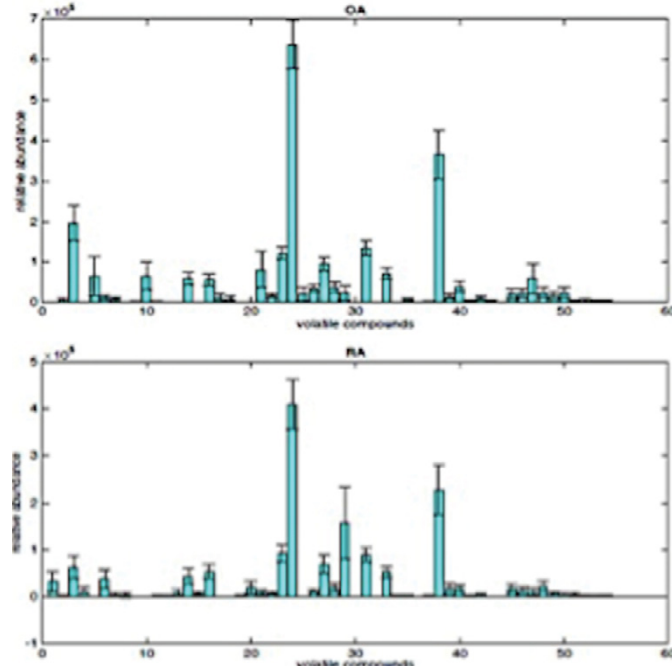
Background: Synovial fluid (SF) receives protein contribution from the tissue around: cartilages, synovial membranes and bones. The presence of inflammation and oxidative stress alters the its chemical composition. In particular, inflammation modulates the release of volatile organic compounds (VOCs) that are the product of reactive oxygen species and free radicals excreted by mitochondria during oxydative stress (1). Articular inflammation plays a major role both in Osteoarthritis (OA) and Rheumatoid arthritis (RA), thus, the identification of specific 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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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.3695

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-inflammatory cytokines (IL-8 and TNF) synergistically increased SF mobility when added together with ACPA. Phosphorylation and inhibition studies of intracellular signalling pathways

in starved SF indicated an important role for PI3K-mediated signals in the ACPA-induced increase of SF mobility.

Conclusions: We demonstrated that additional stimuli (such as stress-induced citrullination and cytokine priming) were needed for SF to react upon ACPA stimulation. This is an indirect proof supporting the idea that a synovial insult that will normally resolve unobserved, might be essential for the transition towards chronic synovial changes in the presence of ACPA.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.6448

FRI0014 ANTIOXIDANT ROLE OF MICROVESICLES FROM ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS IN HUMAN OSTEOARTHRITIC CHONDROCYTES

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Background: Oxidative stress results in the disruption of normal physiologic signaling leading to inflammatory changes, cartilage degradation and osteoarthritis (OA) progression (1). Recent advances have revealed the role of cell-derived microvesicles (MV) as a new mechanism of cell-to-cell communication with potential therapeutic applications. We have shown previously the antiinflammatory effects of human adipose tissue-derived mesenchymal stem cells (AD-MSC) conditioned medium in OA chondrocytes (2).

Objectives: We have isolated the MV fraction from the secretome of AD-MSC to investigate its activity on oxidative stress and inflammation in OA chondrocytes stimulated with interleukin (IL)-1 β . Furthermore, we have characterized the MV protein content by proteomic analysis.

Methods: AD-MSC were isolated from fat of patients who undergone abdominoplasty (without obesity). MV were isolated from AD-MSC conditioned medium by differential centrifugation with size filtration. MV size and concentration were determined by resistive pulse sensing. Proteomic analysis was performed by LC-MS/MS, with ProteinPilot and PeakView software and the bioinformatic tools UNIPROT and PANTHER. OA chondrocytes were isolated from knee specimens of advanced OA patients, stimulated with IL-1 β (10 ng/mL) and treated with MV (3.6x10⁷ particles/mL) for 24h. Accumulation of 4-hydroxy-2-nonenal (HNE)-modified proteins and cytokines were measured by ELISA, NO production and MMP activity by fluorometry. Expression of specific proteins was evaluated by confocal microscopy or immunostaining. The data were analysed by ANOVA followed by Dunnett's test.

Results: MV reduced the accumulation of HNE-modified proteins, a biomarker of oxidative stress-induced lipid peroxidation, in OA chondrocytes stimulated with IL-1 β . The production of NO, IL-6 and TNF α , as well as MMP activity were also significantly reduced by MV treatment, whereas IL-10 and collagen II were enhanced. Proteomic analysis of MV showed high levels (5.89-fold upregulation) of peroxiredoxin 6 (Prdx6), a member of the peroxiredoxin family of antioxidant proteins which is downregulated in OA cartilage (3). MV treatment increased the expression of Prdx6 in OA chondrocytes suggesting a protective role against oxidative stress in these cells.

Conclusions: MV from AD-MSC downregulate the production of oxidative stress and inflammatory mediators in OA chondrocytes. Prdx6, an antioxidant enzyme, may contribute to the protective effects of MV. Our data support the interest of these MV to develop new therapeutic approaches.

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Acknowledgements: Funded by SAF2013-48724-R (MINECO, FEDER) and PROMETEOII/2014/071 (Generalitat Valenciana).

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.3682

FRI0015 MODELLING THE INITIAL PHASE OF FRACTURE HEALING IN VITRO – 3D BONE-LIKE MODELS OF ENDOCHONDRAL OSSIFICATION

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Background: Immunosuppressed patients with ongoing inflammation experience more often difficulties in the process of fracture healing. Hereby amongst others immunomodulated activation of osteoclasts leads to augmented osteolysis. Most notably numerous cytokines and invading lymphocytes provide an inflammatory

environment within the fracture gap, utmost during the first phase of fracture healing. Nowadays researchers, whilst investigating fracture healing, use small rodent models, facing the problem of translation towards the human system. Hence, there is a lack of valid *in vitro* models to examine the first phase of fracture healing. To test new therapeutic strategies, we develop a valid 3D-model using human cells to mimic the first phase of fracture healing *in vitro* which is characterized by the formation of a fracture hematoma, a hypoxic microenvironment as well as inflammation that initiate the healing cascade and the process of endochondral ossification.

Objectives: To develop 3D bone-like models displaying endochondral ossification.

Methods: As a first step, we focus on establishing 3D bone-like, matrix-free models consisting of human mesenchymal stromal cells (hMSC). MSC were isolated from bone-marrow samples of patients undergoing total hip replacement and characterized with regard to their typical surface markers as well as their differentiation potential towards osteogenic, adipogenic and chondrogenic lineage. 3D bone-like models were generated from micro-mass culture of hMSC (Research Center of Medical Technology and Biotechnology, Bad Langensalza). After initial maturation, 3D bone-like models were cultured under hypoxic conditions (37°C, 1% O₂) in osteogenic medium for up to 3 months. *In vitro* μ CT analyses were performed at day 0, after one and two months focusing on the total volume (TV) and bone volume (BV). Additionally osteogenic-relevant genes/factors (Runx2, SPP1, Dlx5, ALP, RANKL, SPI1) as well as exclusion markers (SOX9, PPAR γ 2) were investigated after 3 months of cultivation using qRT-PCR. Finally, we implemented histological/immunohistochemical methods (van Kossa, ALP, Col1 staining).

Results: The 3D bone-like models achieved diameters between 0.5 and 0.7 and a thickness of 0.3 cm. *In vitro* μ Ct analysis revealed a high amount of mineralized tissue at day 0 and substantial increase in the bone volume (BV/TV) after cultivation. At day 0 *in vitro* μ Ct analysis implied mineralization mostly at the margin but penetrated the tissue within further cultivation. These results are also supported by positive van Kossa staining. Additionally, qRT-PCR results yielded higher expressions of *RUNX2*, *SPP1*, *SPI1*, *RANKL* and *DLX5*. Furthermore, immunohistochemistry showed high ALP-activity and Coll-expression.

Conclusions: Preliminary results of our study focusing at developing a 3D bone-like model displayed a promising trend towards modelling endochondral ossification *in vitro* by increased mineralization (*in vitro* μ Ct analysis and van Kossa staining), and upregulation of osteogenic-relevant *RUNX2* and *SPP1* expression as well as ALP-activity and Coll-expression. High expression of *SPI1* and *RANKL* could refer to osteoclast-like activities, which will be in the focus of further investigations. Finally, the complete 3D model will leave us the opportunity for studying the first phase of fracture healing under *in vitro* conditions.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5899

FRI0016 INVOLVEMENT OF RUNX-2 AND β -CATENIN SIGNALING IN THE PRODUCTION OF ADAMTS-7 AND ADAMTS-12 IN OSTEOARTHRITIC SYNOVIAL FIBROBLASTS

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Background: Proteinases released from synovial membrane of osteoarthritis (OA) patients contribute to cartilage damage. We recently reported in synovial fibroblasts (SF) the expression of ADAMTS (a disintegrin and metalloproteinase domain with thrombospondin motifs)-7 and -12, involved in the destruction of the cartilage oligomeric matrix protein (COMP) (1). Signaling pathways regulating these ADAMTS are poorly understood. As Runx2 and β -catenin are two transcription factors involved in chondrocytes metabolism and OA pathology (2–5), we studied whether these factors are implicated in the production of ADAMTS-7 and 12 in SF. Moreover, we analyzed the induction by two inflammatory mediators present in OA joints: interleukin-1 β (IL-1 β), and fibronectin fragments (Fn-fs), previously described in ADAMTS expression (1).

Objectives: To elucidate the signaling pathways involved in the production of ADAMTS-7 and -12 in healthy donors (HD) - and OA-SF.

Methods: ADAMTS-7 and -12 were detected in HD- and OA-SF protein extracts by Western blot. Blockade experiments were performed after stimulation with IL-1 β or 45-kDa Fn-fs. We used inhibitors for two mitogen-activated protein kinases (MAPKs), ERK and p38, implicated in the activation of Runx2, PD98059 and SB203580, respectively. We also used an inhibitor of Wnt/ β -catenin signaling, DDK-1. Levels of ADAMTS-7 and -12 were analyzed by quantitative RT-PCR and ELISA in SF culture supernatants.

Results: Intracellular presence of ADAMTS-7 and -12 was confirmed in HD- and OA-SF, with higher levels of ADAMTS-7 in OA. After IL-1 β or Fn-Fs stimulation, DDK-1 decreased ADAMTS-7 transcript in HD and OA-SF that was translated to a protein reduction in OA. Besides, ERK inhibitor decreased ADAMTS-12 mRNA and protein exclusively in OA-SF.

Conclusions: We reported that ADAMTS-7 protein expression is higher in OA-SF compared to HD confirming previous data at mRNA level (1). As DDK decreased ADAMTS-7, Wnt- β -catenin signaling seems to be implicated in its expression. By contrast, the expression of ADAMTS-12 is regulated by ERK, pointing to a possible implication of ERK-Runx2 axis, exclusively in OA-SF.