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Figure 1. A: Raman spectra (RS) for lesion and adjacent cartilage tissue and assigned peaks; B: Histological stainings (Hematoxylin-eosin (H-E), Safranin-O/Fast-Green (SO-FG) and Toluidin blue (TB)) used for MS analysis from the adjacent and lesion sites from a selected donor; C: rho Spearman's correlation coefficients and significance between RS and MS (total scoring) in lesion and adjacent sites for Observer 1, 2 and 3 (O1, 2 and 3).

FRI0506 ROLE OF MITOCHONDRIA FROM PATIENTS WITH OA IN CELLULAR SENESCENCE

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Background: Chondrorescence, chondroptosis and autophagy contribute to cell death and tissue damage in OA. The mitochondria are related with these three process implicated in the cartilage degeneration. Mitochondrial dysfunction is well documented in OA and has the capacity to promote abnormalities in chondrocyte viability contributing to cartilage degeneration. Cybrids are optimal cellular models to study the mitochondrial function, since they carry different mitochondrial variants with the same nuclear background, therefore, excluding the variations because of nuclear genome.

Objectives: The aim of this work is to study the role of human OA mitochondria in the cellular senescence, apoptosis and autophagy.

Methods: Cybrids were developed using 143B.TK⁺ Rho-0 cell line (nuclear donor) and platelets (mitochondrial donors) from healthy (N) and knee OA donors. Senescence level was measured by real-time PCR method. The percentage of mitochondrial depolarized was evaluated incubating cells with DiIC1(5) in presence of FCCP 1μM using Flow Cytometer. The percentage of apoptotic cells was measured by Flow Cytometry using Annexin-V. Autophagy was evaluated through the developed of Microtubule-associated protein 1A/1B-light chain 3 (LC3) WB. Appropriate statistical analyses were performed with GraphPad Prism v6.

Results: The gene expression corresponding to senescence marker protein (SMP30) showed higher levels in OA cybrids than in N (4.535±1.63; 1.21±0.42 respectively, p<0.0005). OA cybrids showed higher increment depolarized mitochondria under negative stimuli in comparison with basal condition than N (2.57±1.20; p<0.0005; 1.76±0.99; p<0.05 respectively). The analysis of apoptotic levels, when the cells were submitted for a positive stimuli with staurosporine (2 μM) and an inflammatory environment with IL-1β (10 ng/ml), OA cybrids reflected a statistically significant increase in positive cells for Annexin-V in comparison with N cybrids in both conditions (staurosporine 15.68±6.39; 6.41±4.88 respectively, p<0.05. IL-1β 0.924±0.19; 0.47±0.24 respectively, p<0.05). Autophagy was analyzed studying LC3 a marker for autophagosome formation and the results showed that LC3 activation was reduced in OA cybrids (1.19 ±0.24; 1.41±0.21 respectively, p<0.05).

Conclusion: Mitochondria from OA patients is involved in cellular senescence, apoptosis and autophagy (three relevant processes involved in OA).

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FRI0507 THE HUMAN-BASED IN VITRO 3D ARTHRITIC JOINT MODEL

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Background: Our ultimate goal is to develop a valid human *in vitro* 3D joint model to simulate the pathogenesis of arthritis. The model consists of different components including an osteogenic and chondrogenic part, the joint space with synovial fluid and the synovial membrane. It contains all involved cell types and thus, to allow interactions between cells by cell contacts and signaling molecules. The arthritic joint is simulated by the application of sorted immune cells and typical cytokines. As an alternative experimental setup for animal models, our *in vitro* 3D joint model will enable us to study the influence and efficacy of potential drug candidates. Currently, there is no valid 3D model, which is able to mimic an arthritic joint.

Objectives: Here, we aimed to develop the single joint components, namely the osteogenic and chondrogenic part, the joint space with synovial fluid and the synovial membrane, separately and in co-culture without and with the application of neutrophils (PMNs) and cytokines.

Methods: We have used human bone marrow derived mesenchymal stromal cells (hMSCs) to develop the different 3D tissue components that are characterized in detail (e.g. cell vitality, structural integrity) using histological, biochemical and molecular biological methods as well as μCT and scanning electron microscope (SEM). For the osteogenic component, we populated β-tricalcium phosphate (TCP) – mimicking the mineral bony part – with hMSCs, while the scaffold-free 3D cartilage component was generated from a marrow tissue exclusively consisting of hMSC under intermittent mechanical stimulation (fzmb GmbH). Simulating the synovial fluid, non-animal stabilized hyaluronic acid was applied to the osteochondral model. In order to model the synovial membrane, a confluent hMSC layer was formed on a polycarbonate membrane. Subsequently, we applied sorted CD15⁺ cells to the synovial membrane and cytokines (e.g. TNF, MIF, IL6) into the synovial fluid, analyzed using qPCR, multiplex immunoassay and flow cytometry.

Results: We developed an *in vitro* 3D bone model by successfully seeding hMSC on a β-TCP scaffold. Cells consistently adhere onto the scaffold for up to 6 weeks as observed by SEM. The analysis of cell viability via LDH detection and LIVE/DEAD staining showed no toxic effects on the cells even after 3 weeks of incubation as compared to the corresponding control. mRNA expression of bone-related genes such as *RUNX2*, *SPP1* and *COL1A1* as well as μCT analysis confirmed the osteogenic phenotype of hMSC grown in 3D. Mimicking the articular cartilage component, we verified its chondrogenic phenotype by HE and Alcian Blue staining as well as by the reduced mRNA expression of *COL1A1* and an abundant expression of *COL2A1*. Interestingly, co-cultivation of the osteogenic and chondrogenic part looks promising showing a close alignment and cell interaction between both components. Modelling the synovial membrane, we successfully and reproducibly created a confluent hMSC cell layer, with a high cell viability even after 3 weeks of incubation. In addition, we injected cytokines into the synovial fluid. In response, the simulated synovial membrane showed an upregulation of *IL6*, *HIF* and *MMP13* expression on mRNA level. Furthermore, under co-cultural conditions the hMSCs enhanced the survival of the PMNs.

Conclusion: Results from the analysis of the single components confirmed viability, integrity and morphology pointing towards an ultimately successful development of the anticipated *in vitro* 3D model. By combining the different components in a standard 96 well format, we aim to provide a mid-throughput system for preclinical drug testing as well as a valid *in vitro* human-based 3D disease model to study the pathogenesis of arthritis.

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FRIO508 OLIVE OIL POLYPHENOLS AS NOVEL NUTRACEUTICALS IN TREATMENT OF OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is a degenerative disease of the joints characterised by the imbalance of anabolic and catabolic processes in articular cartilage. It is one of the most significant causes of disability in the world affecting over 60% of people over the age of 60, causing joint stiffness, pain and a decrease in the quality of life. Currently no successful disease-modifying agent has been found to prevent or treat the condition. To address this unmet need, alternative approaches, including the use of nutraceuticals as a novel therapeutic intervention are under examination.

Objectives: To analyse if nutraceutical are able to reverse the catabolic activity that contribute to cartilage destruction in OA. Here two polyphenols from extra virgin olive oil (EVOO), oleocanthal (OLC) and ligstroside aglycone (LA), plus a chemically modified acetylated ligstroside aglycone (A-LA), and two marine polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were examined as potential anti-inflammatory agents for OA.

Methods: Human chondrocytes and cartilage explants were extracted from OA femoral heads, cultured and incubated with increasing concentrations of select compounds in combination with the pro-inflammatory cytokines IL-1 β and oncostatin M (OSM). Gene expression levels were analysed by qPCR; nitric oxide (NO), GAG release and cytokines were measured in supernatants by colorimetric assays and Luminex technology respectively. Safranin-O and Alcian blue/Sirius red staining were done to quantify extracellular matrix components from cartilage.

Results: Acetylated ligstroside showed the most promising results for implementation in treating OA as it reduced the expression of pro-inflammatory genes such as inducible nitric oxide (*iNOS*), matrix metalloproteinase-13 (*MMP13*) and interleukin-1 β (*IL1B*) at both RNA and protein levels; decreased nitric oxide (NO) levels from cartilage explants and also reduced proteoglycan (PG) losses in human osteoarthritic cartilage explants and chondrocytes (Figure 1).

Conclusion: These results substantiate the role of nutraceuticals in OA with implications for therapeutic intervention and our understanding of OA pathophysiology.

REFERENCES:

- Castrogiiovanni *et al.* Nutraceutical supplements in the management and prevention of osteoarthritis. *Int. J. Mol. Sci.* 17, (2016).
- Rigacci & Stefani. Nutraceutical Properties of Olive Oil Polyphenols. An Itinerary from Cultured Cells through Animal Models to Humans. *Int. J. Mol. Sci.* 17, 1–28 (2016).
- Aparicio-Soto, *et al.* Extra virgin olive oil: a key functional food for prevention of immune-inflammatory diseases. *Food Funct.* 7, 4492–4505 (2016).

Figure 1. Stimulation with A-LA recovered proteoglycan (PG) losses. OA cartilage punches were cultured for 14 days in different conditions then sectioned to show the top superficial layer down to the deep zone at the bottom. Safranin-O staining visualised PG showing how exposure to the inflammatory environment of IL-18/OSM caused losses to PG that were recovered with A-LA 10 μ M but worsened with higher concentrations. Same results were observed with Alcian blue for PG and Sirius Red for collagen in matrix. All scale bars represent 200 μ m.

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FRIO509 3D MICROSTRUCTURE OF INTACT AND OSTEOARTHRITIC HUMAN MENISCUS USING MICRO-COMPUTED TOMOGRAPHY

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Background: Degenerative meniscal lesions are highly prevalent in the general population and are associated with increased risk of knee osteoarthritis (OA). Development of novel methods for visualization of the early degenerative changes in the meniscus could help us better understand meniscal degradation and the onset of OA.

Objectives: To develop and perform *ex vivo* 3D imaging of meniscus posterior horn microstructure with micro-computed tomography (μ CT), and to compare specimens from healthy references with end-stage OA using histology and qualitative μ CT.

Methods: Medial and ipsilateral lateral menisci were retrieved from 10 patients undergoing total knee arthroplasty for medial compartment knee OA (age range 50 to 75 years, 5 women). Additionally, medial menisci were obtained from 10 cadaveric donors without known OA, to serve as reference (age range 18 to 77 years, 5 women). The samples were freshly frozen in -80°C. After thawing, the posterior horns were dissected and fixed, followed by excision of a piece for μ CT imaging (Fig. 1A). The μ CT samples were dehydrated in ascending ethanol concentrations, treated with hexamethyldisilazane (HMDS), and dried in room temperature. They were then imaged with a desktop μ CT (scanning parameters: 40 kV, 250 μ A, 2.0 μ m pixel size, 1815 ms, no additional filtering). For histological analysis, Pauli's histopathological scoring¹ was performed independently by two graders on sections from three regions (Fig. 1A). The graders' consensus was given for each sub-score, from which the final overall consensus scores were calculated for each region. The mean difference in consensus scores (95% CI), adjusted for age and sex, were estimated between the menisci from OA knees and the reference menisci (medial OA menisci vs reference menisci, and lateral menisci from OA patients vs reference menisci) using a mixed linear regression model. The mean difference in consensus scores between the medial and lateral menisci from the same OA patient was calculated using a linear fixed effects model. The models were fitted on all three histological regions, but here we report histology results from region 2, because this region is adjacent to the piece analyzed by μ CT (Fig. 1A).

Figure 1. A: Wedge-shaped sections were cut from the meniscus posterior horn for μ CT analysis and histological scoring (regions 1, 2 and 3). B: The histological consensus scores from region 2 are plotted against the subject's age in the three sample groups (lateral, medial OA, reference). The mean (SD) consensus scores of each group: lateral menisci from OA patients: 9.5 (2.5); medial OA: 14.1 (1.7); reference: 8.5 (3.5). C: Volumetric visualizations of menisci representing Pauli's meniscus histopathological grades 1-4 imaged using HMDS-based μ CT (top row), and their adjacent Safranin-O-Fast Green-stained histological sections (bottom row). It can be seen that the same structural features, such as surface morphology, collagen organization, and tissue calcification, can all be visualized in 3D with μ CT. Grade 1 (scores 0-4): normal tissue. Grade 2 (scores 5-9): mild degeneration. Grade 3 (scores 10-14): moderate degeneration. Grade 4 (scores 15-18): severe degeneration.

Results: 3D visualization of the meniscus with μ CT revealed similar structural changes as histological analysis (Fig. 1C). The histopathological consensus scores were generally higher in medial OA samples as compared to both lateral menisci from OA patients and the reference menisci (Fig. 1B). The mean difference in histological scores (95% CI) was 4.3 (2.5, 6.1) for medial OA menisci vs reference menisci, -0.3 (-2.2, 1.5) for lateral menisci from OA patients vs reference menisci, and 4.6 (3.0, 6.2) for medial OA menisci vs lateral menisci from OA patients.

Conclusion: An HMDS-based 3D μ CT protocol allows unique 3D visualization of meniscus microstructures. The method enables the visualization of considerably large volumes and evaluation of the tissue's 3D