PRESENTATION NUMBER: LBA-47
CHONDROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS MEDIATED BY EXTRACELLULAR VESICLES FROM PLASMA- AND SERUM-BASED BLOOD-DERIVED PRODUCTS FOR OSTEOARTHRITIS THERAPY

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Purpose: Plasma- or serum-based blood-derived products are intra-articularly injected into osteoarthritic joints to favour pain relief, reduce inflammation and promote cartilage regeneration. Preparations of platelet-rich plasma anti-coagulated with citrate (CPRP) are often applied, but also anticoagulant- and cell-free alternatives such as hyperacute serum (hypACT) are investigated. In addition to growth factors, blood products contain extracellular vesicles (EV) which are around 30-1000 nm sized membraneous particles and are carriers of signal molecules such as lipids, proteins or RNAs. These open up new levels of complexity at understanding mechanisms of action of blood products. Therefore, this study outlines roles of EVs isolated from blood products and characterises their contribution to the regenerative potential of these blood products.
Methods: To attribute potential chondroprotective and anti-inflammatory effects elicited by the EV fraction of blood products, ultracentrifugation was used to enrich EV from blood products. Concentration and mode size of enriched EVs was assessed via nanoparticle tracking analysis (NTA). Presence of EV marker proteins and depletion of frequently co-isolated components such as lipoproteins was monitored via Western Blot. Primary OA chondrocytes were then treated with the EVs in presence or absence of IL1β. Gene expression changes were analysed via reverse transcription quantitative PCR (RT-qPCR) and Western Blot. Cytokine release was monitored via enzyme-linked immunosorbent assay (ELISA) in sandwich format.
Results: Gene expression analysis revealed increased levels of type II collagen (COL2A1), SRY-box transcription factor 9 (SOX9) and aggrecan (ACAN) compared to full blood products, but also of the catabolic marker and tissue remodeling factor matrix metalloproteinase 3 (MMP3). hypACT EVs prevented increased type I collagen (COL1A1) expression compared to CPRP EVs. CPRP blood product increased SOX9 protein expression, whereas CPRP EVs prevented SOX9 expression and decreased COX2 levels. In contrast, hypACT EVs elevated COX2 expression, while promoting SOX9 expression. Enzyme-linked immunosorbent assay (ELISA) targeting IL6 released from chondrocytes showed a dramatic decrease in presence of EVs from hypACT or CPRP compared to the respective blood products.
Conclusions: The Results indicate that blood EVs are sufficient to promote chondrogenic gene expression changes in OA chondrocytes, while preventing pro-inflammatorsy cytokine release compared to full blood products. This highlights the potential of blood-derived EVs as regulators of cartilage extracellular matrix metabolism and inflammation as well as candidates for new cell-free therapeutic approaches for OA.

PRESENTATION NUMBER: LBA-48
ARTICULAR CARTILAGE REGENERATION BY ACTIVATED SKELETAL STEM CELLS

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Purpose: Here we investigate the ability of resident skeletal stem-cell (SSC) populations to regenerate cartilage in relation to age, a possible contributor to the development of osteoarthritis (OA).
Methods: We tested if microfracture (MF) surgery could stimulate expansion of SSCs on the chondral surface of adult limb joints in mice by inducing a localized regenerative response. We then tested if exogenously applied factors could skew the differentiation of the activated SSCs towards hyaline cartilage.
Results: We find that although activated SSCs tended to form fibrous tissues, localized co-delivery of BMP2 and soluble VEGFR1 (sVEGFR1), a VEGF receptor antagonist, in a hydrogel skewed differentiation of MF-activated SSCs towards articular cartilage.
Conclusions: These data indicate that following MF, a resident stem-cell population can be induced to generate cartilage for treatment of localized chondral disease in OA.

PRESENTATION NUMBER: LBA-49
THROMBOCYTE DERIVED PRODUCT EFFICACY IN KNEE PTOA TREATMENT

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Purpose: to study efficacy and safety of the platelet autologous plasma (PAP) comparing to standard treatment in young patients with symptomatic knee post traumatic OA (PTOA)
Methods: Study included 62 patients (mean age 38.29±3.51 years) with established symptomatic knee PTOA (mean time from trauma - 46.87±2.09 months), I-II stages (X-ray). Patients with prominent known primary OA risk factors (obesity, metabolic diseases), after knee surgery, with other arthritis or any other uncontrolled diseases and disorders were not included in the study. All patients were concented to participate in the study and were divided into 2 groups -Gr.1 received standard treatment (NSAIDs, exercises, multimodal physiotherapy), Gr.2 - received the course of 3 intra-articular injections of PAP in addition to the standard treatment. Efficacy and safety was evaluated by KOOS, VAS, laboratory investigations.
Results: During early observation period (first 2 weeks) all patients with PTOA demonstrated significant improvement in pain and functional activity, comparing to baseline values but patients from Gr.2 demonstrated better daily living activities and better points in KOOS sport and recreation subscales. In 4 weeks the difference between groups were more prominent, with better results of treatment in group treated with PAP. Later, during the late observation period, second group still demonstrated better outcomes (both comparing to the baseline and to the first group), while Gr. 1 patients partly returned to the baseline levels; after 3-6 months one third of Gr. 1 patients experienced 1-2 OA exacerbations, accompanied by repeated NSADs use; in Gr.2 only 6.45% of patients has had 1 OA exacerbation (p&l0.05). At 12 months period the majority of Gr.2 patients still had better knee functional capacity and less pain comparing to the baseline, while Gr.1 patients showed no significant difference with baseline in all KOOS parameters. No significant complications were observed during PAP use, except of temporary local pain at the injection site.
Conclusions: Use of the PAP intraarticular injections in addition to the standard PTOA treatment improves both early and late Results of treatment, decreases the number of OA exacerbations and need in NSAIDs use during 12 months after treatment.

PRESENTATION NUMBER: LBA-50
THERAPEUTIC MOLECULES FOR OSTEOARTHRITIS TREATMENT. ROLE OF PHLORETIN, IPRIFLAVONE AND RALOXIFENE IN LIPOPOLYSACCHARIDE INDUCED OSTEOARTHRITIC CHONDROCYTES

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Purpose: The search of novel molecules for the treatment of osteoarthritis (OA) is complex as any new therapeutic approach should encompass these requirements: inhibition of cartilage degradation, protection of bone and inhibition of inflammation. In the last years, different drugs have been proposed though most of them did not succeed in fulfil these requirements. Moreover, few of them have been encapsulated in drug delivery systems to improve their therapeutic potential to achieve a sustained or controlled release compared to the administration of equivalent doses of the free compounds. Nanoscience has arisen in the last decades as a potential field of study in drug delivery because nanomaterials may overcome the main current limitations to achieve an efficient and localized drug delivery by improving the targeted delivery and providing a sustained or controlled delivery to prolong the therapeutic effect. On the other hand, different polyphenols and aromatic organic compounds are known to possess anti-

inflammatory, antioxidant and bone density-building properties. However, their potential for treating OA has not been widely explored. The aim of this study was to evaluate the anti-inflammatory potential of 3 compounds of this nature (phloretin, ipriflavone, raloxifene) and their capacity to attenuate cartilage degeneration on human osteoarthritic chondrocytes standing alone or when encapsulated within polymeric nanoparticles.

Methods: Patient's samples were obtained from total knee replacement surgeries performed in the Hospital Clínico Universitario Lozano Blesa (Zaragoza, Spain). Patient's consent and ethics committee approval (PI20/429) were granted by the Aragon Government. Primary human articular chondrocytes were obtained from cartilage slices cut from macroscopically osteoarthritic cartilage of the replaced knees. The slices were finely cut and digested in a 1:1 mixture containing DMEM-F12 and 0.25% trypsin. Then, samples were centrifuged and further digestion was carried out overnight at 37 ºC with 0.02% type II collagenase. Subsequently, chondrocytes were filtered and seeded at a cell density of 105 cells per flask of 25 cm2 in DMEM-F12 supplemented with 10% FBS, 1% antibiotics-amphotericin, 1% glutamine, 5 ng/mL FGF-2 and 5 μg/mL insulin. When cells were confluent, cells were passaged and the experiments were performed in early passages (passages 1 and 2). First, cell viability was evaluated after treatment with phloretin (10-100 μM), ipriflavone (10-100 μM) and raloxifene (0.1-10 μM) for 24 and 48 h. These compounds were dissolved in supplemented medium containing 1% ethanol except for raloxifene (0.1% DMSO). After treatment, the Blue Cell Viability Assay was developed by adding 10% of the reagent to the supplemented medium. After incubation, viability was evaluated by fluorescence reading in a microplate reader (530 nm excitation/590 nm emission). Then, apoptosis was studied after treatment with the mentioned compounds and also as a pretreatment of cells for 2 h prior to cell stimulation with lipopolysaccharide (LPS, 1 μg/mL) for 24 h to mimic inflammation conditions. After treatment and incubation, cells were washed and centrifuged to be then resuspended in PBS at final concentration of 106 cells/mL. Subsequently, cells were centrifuged and the pellet was resuspended in Annexin-binding buffer and stained with Annexin V-FITC. On the other hand, type II collagen and aggrecan markers were evaluated through the kit Intracell together with secondary antibodies (Alexa 488 and 683, respectively) to elucidate the potential effects of the treatment with the proposed compounds in cartilage biomarkers. Then, the samples obtained from both assays (apoptosis and cartilage biomarkers) were analyzed by flow cytometry in a Gallios equipment and Kaluza Software.

Results: The results obtained showed a great potential for the molecules analyzed in OA treatment. The encapsulating polymer of choice was the FDA approved poly(lactic-co-glycolic acid) (PLGA) using single emulsion solvent evaporation to take advantage of the hydrophobic nature of those compounds assuring high drug loadings. Cell viability (Fig 1) displayed percentages higher than 70% in all cases, fulfilling the recommendations of the ISO 10993-5. Cell viability percentages were concentration-dependent and also higher after treatment for 48 h compared to those obtained after treatment for 24 h, which suggests the degradation of the compound due to cell metabolism. Cell apoptosis was studied both in cells treated with the compounds (Fig 2) and also in cells pretreated for 2 h with the compounds and stimulated with LPS for 24 h (Fig 3). In these studies, diclofenac (100 μM) was used as positive control for the reduction of inflammation. All the figures shown depict the results obtained when treating cells with phloretin though all the tested compounds displayed similar trends. Fig 2 displays the results obtained when cells were treated with phloretin. The treatment with this compound showed high viability percentages and apoptosis or necrosis were not increased with the treatment though diclofenac involved a slight increase (< 5%) in apoptosis (early + late) compared to the control sample. The treatment with LPS was carried out at a concentration of 1 μg/mL as previously indicated in the literature. In fact, this concentration did not involve apoptotic effects, pointing to its suitability for inflammation induction. In this sense, the pretreatment with the compounds and subsequent inflammation induction did not yield changes in cell apoptosis profile, which is very relevant for their further application. Finally, characteristic cartilage biomarkers (aggrecan and type II collagen) were also analyzed by flow cytometry (Fig 4). These results showed a very slight decrease in type II collagen together with a slight increase in aggrecan concentration when LPS was added compared to the not treated sample. When phloretin was added to the cells, a significant decrease in aggrecan concentration was found at the highest concentration assayed, whereas the lowest concentrations exerted closer percentages to the control sample, pointing to these lower doses as the most adequate for our intended application to encapsulate the compounds and prolong their effect.

Conclusions: Our studies highlight the suitability of phloretin, ipriflavone and raloxifene to be encapsulated in polymeric nanoparticles and prolong their therapeutic effect as these compounds did not show detrimental effects in primary osteoarthritic chondrocytes. With this scenario, it seems interesting to develop long-lasting drug delivery nanosystems able to release those anti-inflammatory active principles and evaluate their potential use in OA treatment.

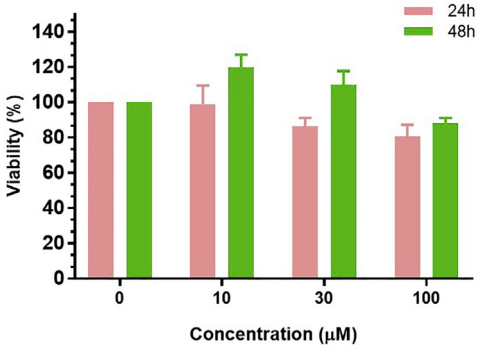

Figure 1. Percentage of human osteoarthritic chondrocytes viability after exposure to phloretin at different concentrations (10-100 μM) for 24 and 48 hours. The cell viability was determined by the Cell Blue Viability assay. Data are expressed as the mean ± SEM (n = 5).

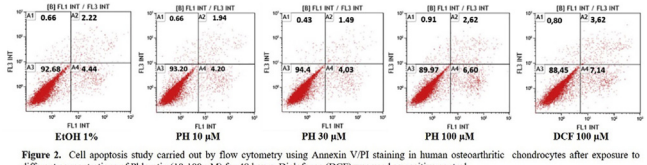

Figure 2. Cell apoptosis study carried out by flow cytometry using Annexin V/PI staining in human osteoarthritic chondrocytes after exposure to different concentrations of Phloretin (10-100 μM) for 48 hours. Diclofenac (DCF) was used as positive control.

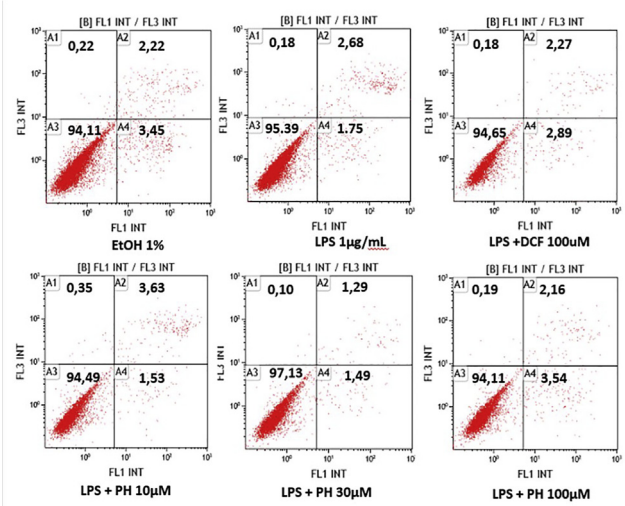

Figure 3. Effect of the treatment with phloretin on LPS-induced human osteoarthritic chondrocytes. Cells were pretreated for 2 hours with different concentrations of phloretin (10-100 μM) and then stimulated or not stimulated with LPS (1 μg/mL) for 24 hours. Diclofenac 100 μM was used as positive control to alleviate inflammation.

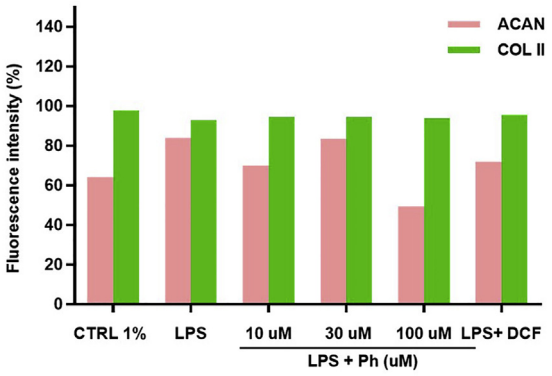

Figure 4. Percentage of fluorescence intensity obtained from flow cytometry assays of biomarkers aggrecan (ACAN) and collagen type II (COL II) when treating with phloretin LPS-induced human osteoarthritic chondrocytes. Cells were pre-treated for 2 hours with different concentrations of phloretin (10-100 µM) and then stimulated with LPS (1 ug/mL) for 24 hours. Diclofenac 100 µM was used as positive control to alleviate inflammation.

PRESENTATION NUMBER: LBA-51
ADHERENCE TO THE MEDITERRANEAN DIET IN PATIENTS WITH OSTEOARTHRITIS AND HEALTHY SUBJECTS USING THE MEDAS QUESTIONNAIRE

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Purpose: The Mediterranean diet (MD) has proven beneficial in a large number of chronic diseases. The relationship between the MD and rheumatic diseases is complex and there are few studies that have studied this relationship. These show that there could be a positive association between adherence to the Mediterranean diet (MD-A) and a lower prevalence of OA.The aim of the study is to determine MD-A and whether there is a different pattern of consumption in patients with OA and healthy subjects.
Methods: Observational, cross-sectional and multicenter study. Patients attending the rheumatology consultation and meeting the ACR criteria for OA of the hands, knees, or hips were included in the study. The healthy ones were recruited from the health personnel and companions of patients who do not live in the same address as the patient or have a diagnosed rheumatic disease. The study was carried out in the rheumatology consultation of two Hospitals (one tertiary level and the other regional) and an outpatient center with specialized care. All participants included in the study have answered a survey of 14 questions (MEDAS-14), based on the Predimed study, which assesses MD-A. Fisher's exact test and Mann-Whitney U test have been used to evaluate the statistical significance of each question (qualitative and quantitative analysis). The study was approved by the Ethics and Clinical Research Committee of the centers.
Results: 162 surveys were conducted, 90 responded by patients with OA and 72 by healthy participants. Patients with OA have less adherence to MD than healthy ones, but this difference is not statistically significant (6.86 vs 7.14). However, in 7 of 14 questions we found statistically significant differences. The proportion of patients with OA who consume 2 or more servings of vegetables per day is lower than that of healthy subjects (14% vs 33%, p <0.05). These differences were also significant in the quantitative analysis (0.79 vegetables/day vs. 1.26; p <0.05). The consumption of more than three fruits a day is more frequent in OA than in healthy ones (44% vs 28%, p <0.05). The quantitative analysis is also significant (2.19 vs 1.88, p <0.05). OA patients consume more butter than healthy people (81% of OA patients eat less than 1 serving of butter per day vs. healthy 99%, p <0.05). The quantitative analysis is also significant (0.20 vs 0.01, p <0.05). Patients with OA consume fewer glasses of wine than healthy ones (0.52 vs 1.25, p <0.05). The proportion of OA that consumes 3 or more servings of legumes per week is lower than that of healthy ones (9% vs 21%, p <0.05). The proportion of OA that eats more than 3 weekly servings of nuts compared to healthy ones is also lower (19% vs 38%, p <0.05). The quantitative analysis is also significant (2.31 vs 1.30, p <0.05). The proportion of patients with OA who preferentially consume white meat over red is higher than in healthy subjects (92% vs 79%, p <0.05).
Conclusions: There are different patterns of food intake evaluated in the MEDAS-14 questionnaire between patients with OA and healthy people. OA patients eat fewer vegetables, fewer legumes, fewer glasses of wine, and fewer nuts, but their butter intake is higher. However, OA patients eat more fruit and more white meat than red than healthy participants. The MD-A diet quantified by MEDAS-14 in subjects with OA is lower than in healthy participants, but this difference is not statistically significant. Longitudinal intervention studies are necessary to assess whether the differences observed in this study have any causal relationship.

PRESENTATION NUMBER: LBA-52
MASS SPECTROMETRIC DETERMINATION OF THE PEPTIDE PROFILE OF GENACOL®, A HYDROLYSED COLLAGEN-BASED NUTRITIONAL SUPPLEMENT

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Purpose: Osteoarthritis (OA) is the most common form of arthritis and a leading cause of pain and physical disability across the world. OA affects millions of patients and places a substantial economic burden on health and social care systems. The prevalence of OA continues to grow due to the obesity epidemic and the increasing size of the aging population. In the absence of disease-modifying drugs and effective treatments, OA is expected to carry an even greater economic and social burden in the future. Therefore, considering the numerous challenges associated with the treatment of OA, there is an urgent need to identify new opportunities for the development of preventive and interventional strategies for the management of OA. Nutritional supplements have the capacity to support joint health and alleviate OA symptoms, restoring quality of life to patients. Many other medical disciplines and societies are already practicing multimodal treatments, including the use of nutritional supplements. Recent studies have shown that oral administration of low-molecular-weight collagen-derived supplements Results in increased postprandial plasma concentrations of amino acids. It is also known that enzymatic hydrolysis of collagen derivatives increases the absorption rate and bioavailability of glycine, proline, and hydroxyproline. However, there have been no studies on the molecular composition of commercially available collagen hydrolysates. The aim of this study was to determine the molecular composition and peptide mass distribution pattern of Genacol® capsules, an innovative nutritional supplement containing collagen hydrolysates.
Methods: We determined the molecular composition and peptide mass distribution pattern of three different formulations of Genacol® using reverse phase chromatography coupled to electrospray ionization-quadrupole time of flight spectrometer (LC-ESI-QTOF). This analytical technique combines UHPLC separation and mass analysis by tandem mass spectrometry, a widely used analytical tool for determining the molecular weight and identification of biological molecules, in this case, proteins and their constituent peptides. Ten milligrams of Genacol® powder were dissolved in 1 ml of water + 0.1% formic acid, prior to injection in the UHPLC system and mass spectrometric analysis. A semi-