(score 3, range 1.5-15), and sclerotic SB area was higher in the Med condyles (64.3 $\pm$ 7.0 vs 27.6 $\pm$ 7.4%, p<0.05). CD271+ MSCs were found in bone-lining and perivascular locations, and accumulated in the areas of vascular invasion into the articular cartilage in both, Med and Lat condyles. No differences in the MSC numbers were found using CFU-F assay (1.4 $\pm$ 0.7 and 1.6 $\pm$ 0.8% of total cells in Lat and Med condyles, respectively), or by flow cytometry (4.4 $\pm$ 3.9 and 5.9 $\pm$ 6.8%, respectively). MSCs had similar PD and trilineage capacities *in vitro*. Out of 95 gene tested, 3 genes were significantly upregulated in Med condyle CD271+ MSCs: GREM1 (lateral MSCs below detection), PTHLH (2.4-fold, p=0.02) and STMN2 (10.5-fold, p=0.02), all implicated in osteogenic differentiation and mineralisation (6, 7, 8).

**Conclusion:** Upregulation of ossification-related genes in Med condyle MSCs suggest their potential contribution to sclerotic plate formation and OA severity. Further work is needed to establish if biomechanical or biological stimulation of these MSCs can result in GE modulation in preference of cartilage tissue formation and subchondral bone restoration.

### REFERENCES

- [1] Conaghan. Nat Rev Rheumatol (2013).
- [2] Zhen. Nat Med (2013);
- [3] Campbell. Arthritis Rheumatol (2016);
- [4] Bae. Cartilage (2010).
- [5] Altaie. Cytotherapy (2018);
- [6] Hu. Mol Med Rep (2017);
- [7] García-Martín. Mol Endocrinol (2014);
- [8] Chiellini. Biochem Biophys Res Commun (2008)

Acknowledgement: CSR funded by fellowship Xunta de Galicia (Consell de Cul, Educ e Ordenación Univ). TB and DM part-supported by NIHR Leeds Musculoskeletal Biomed Research Centre. TB part-supported by FOREUM. JE part-funded by AO foundation start-up grant.

Disclosure of Interests: Clara Sanjurjo Rodriguez: None declared, Thomas Baboolal: None declared, Agata Burska: None declared, Frederique Ponchel: None declared, Jehan El-Jawhari: None declared, Joseph Aderinto: None declared, Owen Wall: None declared, Hermant Pandit Grant/research support from: GSK, Consultant for: For education for Bristol Myers Squibb, Dennis McGonagle Consultant for: Lilly, Novartis UCB, Speakers bureau: Lilly, Novartis UCB, Elena Jones: None declared

DOI: 10.1136/annrheumdis-2019-eular.2649

#### AB0099 COCULTURE OF SYNOVIAL MEMBRANE AND CARTILAGE: A POTENTIAL HUMAN EX VIVO CARTILAGE DEGRADATION MODEL

Johanna Mucke<sup>1</sup>, Victoria Warzynski<sup>1</sup>, Ellen Bleck<sup>1</sup>, Thomas Pauly<sup>2</sup>, Tim Claßen<sup>2</sup>, Matthias Schneider<sup>1</sup>, Stefan Vordenbäumen<sup>1</sup>. <sup>1</sup>Policlinic for Rheumatology & Hiller Research Centre for Rheumatology, Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany, <sup>2</sup>Dept. of Orthopdedic Surgery, St. Elisabeth-Hospital, Rheinisches Rheumazentrum, Meerbusch-Lank, Germany

**Background:** Cartilage degradation is a hallmark in both osteoarthritis (OA) and rheumatoid arthritis (RA). Ex vivo cartilage models offer the potential to study disease processes but are surprisingly rarely used.

**Objectives:** Here we investigate the effect of synovial tissue and stimulation with TNF- $\alpha$  on cartilage degradation in a physiologically relevant coculture-model of synovial membrane and cartilage.

**Methods:** Cartilage and synovial membrane were collected from patients with RA and OA who underwent joint replacement. Cartilage was cultivated either alone or with synovial membrane in medium with or without TNF $\alpha$  as a stimulant for up to 28 days. The explants were harvested at different time points and Safranin-O-staining was performed to visualize proteoglycan (PG) content. A scoring system from 0 (no PG content) to 3 (high PG content) was applied as a semi-quantitative measure for evaluation. Linear mixed modeling adjusted for gender and age was performed to identify factors influencing PG content.

**Results:** Samples of 18 patients (9 OÅ, 9 RA) were collected. Factors associated with proteoglycan loss in cartilage were the time in culture (p=0.02), stimulation with TNF- $\alpha$  (p=0.0007) and coculture with synovial membrane (p=0.00002) in univariate analysis with TNF- $\alpha$  (p=0.0009) and coculture (p=0.001) maintaining the level of significance in multivariate analysis. (Figure 1)

**Conclusion:** Exposure to synovial tissue and TNF stimulation drive cartilage proteoglycan loss in an ex vivo cartilage degradation model. This model may prospectively serve to investigate pathophysiologic pathways of cartilage degradation in both OA and RA.

## REFERENCES

None

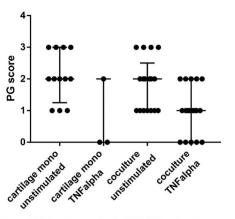


Figure 1. Proteoglycan content at d38. PG, proteoglycan.

Disclosure of Interests: Johanna Mucke: None declared, Victoria Warzynski: None declared, Ellen Bleck: None declared, Thomas Pauly: None declared, Tim Claßen: None declared, Matthias Schneider Grant/research support from: GlaxoSmithKline and UCB Pharma for performing the LuLa-study., Speakers bureau: Chugai, Stefan Vordenbäumen: None declared DOI: 10.1136/annrheumdis-2019-eular.2159

#### AB0100 COMPARATIVE ANALYSIS OF THE EFFECTIVENESS AND TOLERANCE OF INTRA-ARTICULAR INJECTION OF PLATELET RICH PLASMA VS HYALURONIC ACID AND NON-ARTHROSCOPIC JOINT LAVAGE IN PATIENTSWITH KNEE OSTEOARTHRITIS

Rafaela Ortega Castro<sup>1</sup>, Rocio Segura<sup>1</sup>, Jerusalem Calvo Gutierrez<sup>1</sup>, Rocio Espino-Garcia<sup>2</sup>, María del Carmen Castro Villegas<sup>1</sup>, Alejandro Escudero Contreras<sup>1</sup>, Miguel A. Caracuel-Ruiz<sup>1</sup>, Ladehesa Pineda Lourdes<sup>1</sup>, Desiree Ruíz-Vilchez<sup>1</sup>, Montserrat Romero-Gómez<sup>1</sup>, Pilar Carreto-Font<sup>3</sup>, Eduardo Collantes Estevez<sup>1</sup>, Font Ugalde Pilar<sup>1</sup>. <sup>1</sup>*Reina Sofía University Hospital/ Maimónides Institute of Biomedical Research of Córdoba (IMIBIC);*, Clinical *Management Unit of Rheumatology, Córdoba, Spain;* <sup>2</sup>*University of Cordoba/Reina Sofia Hospital, Córdoba, Spain;* <sup>3</sup>*Tudela Duero Clinic/East Area, Valladolid, Spain* 

**Background:** Knee Osteoarthritis (KOA) is a common condition associated with pain and morbidity. The impact of this pathology makes it necessary to develop new procedures for joint regeneration. Regenerative treatments such as plateletrich plasma (PRP) are being proposed as a safe alternative, capable of regenerating damaged tissues and improving the quality of life, reducing the need to surgical procedures (1-4).

**Objectives:** To compare the effectiveness of PRP treatment vs Hialuronic Acid (HA) and Non-Arthroscopic Joint Lavage (NAJL) in patients with KOA.

**Methods:** It was performed a prospective, 3-months observational study of 51 patients with KOA, from the OA clinic of the Reina Sofía Hospital of Córdoba. Patients were treated according to clinical practice in 1 of the 3 treatment arms (PRP, HA, NAJL). Pain and disability were evaluated using Visual Analogue Scale (VAS) of joint pain, WOMAC and OARSI. We compared the baseline data using simple ANOVA and chi-squared tests and the intra- and inter-group differences before and after treatment (0, 1 and 3 months) using the mixed ANOVA test. All comparisons were bilateral considering p<0.05 as a significant result.

**Results:** 51 patients were enrolled, of which 52.9% were female. The average age (±SD) was 58.47 ± 7.48 years. The average Body Mass Index (BMI) was 30.64 ± 4.25 Kg/m<sup>2</sup>. Characteristics at baseline were comparable among the 3 treatment groups (Table 1). An overall improvement was observed during the follow-up in the PRP group in terms of pain and disability with respect to the baseline, with a VAS MD of -3.00 [95%, CI, -4.79 to -1.21] in the first month and -3.08 [CI 95%, -4.79 to -1.38] at 3 months (p < 0.001), a MD WOMAC of -18.83 [95% CI, -30.62 to -7.05] (p = 0.001) in the first month, and a MD of OARSI of -19.32 [95%, CI, -34.26 to -4.37], (p = 0.008) in the first month and -18.94 [95% CI, -36.81 to -1.07] at 3 months (p = 0.035). In addition, the PRP group was superior to the HA group, showing a significant improvement in the VAS at 1 and 3 months (p = 0.005) and compared to the NAJL group, PRP showed better results in the WOMAC throughout the follow-up (p < 0.01) and in the OARSI at 3 months (p = 0.013).

**Conclusion:** Based on our results, the intra-articular infiltration with PRP could therefore constitute a safe and effective treatment option in KOA patients, with short-medium term results superior in terms of pain and disability to conventional treatment with HA and NALJ.

Table 1. Baseline characteristics of patients:

Variable	PRP group (n=16)	HA group (n=22)	NALJ group (n=13)	Ρ
Age (years) (x±SD)	59.56±8,70	63.32±5,49	62.23±7.73	NS*
BMI (kg/m <sup>2</sup> ) (x±SD)	30.31±3.75	30.3±3.36	31.99±5.94	NS*
Female (n,%)	8(50)	12(54.5)	7(53.8)	NS**
KOA stage (n,%)				NS**
11	5(33.3)	11(50)	6(46.2)	
	10(66.7)	11(50)	7(53.8)	
Knee treated (n,%)				NS**
Left	4(25)	12(54.5)	7(58.3)	
Right	12(75)	10(45.5)	5(41.7)	
VAS pain (x±SD)	6.81±2.14	6.73±1.55	6.54±2.07	NS*
WOMAC (x±SD)	48.56	48.68	57.69±18.98	NS*
	±13.31	±16.09		
OARSI (x±SD)	63.92	57.03	70.80±13.51	NS*
	±17.48	±18.78		

NS: not significant. statistical significance based on: \*the simple ANOVA test and \*\* on the Xi-square test.

#### REFERENCE

 Huang Y, et al. Orthopade. 2019 Jan 8. 2)Burke, et al. Clin trans Med (2016) 5-27. 3)Wroblewski AP, et al. Oper Tech Orthop 2010; 20: 98-105.
4) De La Mata J, et al. Reumatol Clínica. 2013; 9(3):166-71.

**Disclosure of Interests:** Rafaela Ortega Castro: None declared, Rocio Segura: None declared, Jerusalem Calvo Gutierrez: None declared, Rocio Espino-Garcia: None declared, María del Carmen Castro Villegas Paid instructor for: MSD, Abbvie, Pfizer, Janssen, Lilly, Roche, Alejandro Escudero Contreras: None declared, Miguel A. Caracuel-Ruiz: None declared, Ladehesa Pineda Lourdes: None declared, Desiree Ruíz-Vilchez: None declared, Montserrat Romero-Gómez: None declared, Pilar Carreto-Font: None declared None declared, Font Ugalde Pilar: None declared **DOI:** 10.1136/annrheumdis-2019-eular.6598

AB0101 SECRETOME ANALYSIS OF CHONDROCYTES AND SYNOVIAL FIBROBLASTS IN OSTEOARTHRITIS: MODULATION BY VIP

Selene Pérez García<sup>1</sup>, Valentina Calamia<sup>2</sup>, Tamara Hermida Gómez<sup>2</sup>, Mar Carrión<sup>1</sup>, Raúl Villanueva Romero<sup>1</sup>, Yasmina Juarranz<sup>1</sup>, Francisco J. Blanco<sup>2</sup>, Rosa P. Gomariz<sup>1</sup>, on behalf of Bases moleculares y celulares de enfermedades inflamatorias/autoinmunes. Departamento de Biología Celular. Facultad de Biología, UCM. Grupo de Investigación en Reumatología, Instituto de Investigación Biomédica de A Coruña (INIBIC).. <sup>1</sup>Facultad de Biología, Universidad Complutense de Madrid (UCM), Departamento de Biología Celular, Madrid, Spain; <sup>2</sup>Instituto de Investigación Biomédica de A Coruña (INIBIC), Servicio de Reumatología, A Coruña, Spain

**Background:** Osteoarhthritis (OA) is a chronic, degenerative and multifactorial disease, and the main cause of pain and dysfunction among elder people. It is characterized by a progressive loss of function of synovial joints (1). The role of chondrocytes in this pathology has been widely studied (2), but other joint cells are also involved, including the synovial fibroblast (SF) (3-5). During joint destruction, inflammatory and degradative mediators are released by joint cells and from the extracellular matrix (ECM), including fibronectin degradation fragments (Fn-fs) (3, 4, 6). On the other hand, vasoactive intestinal peptide (VIP) exerts anti-inflammatory disorders, including OA (3, 5). The study of the mediators released by joint cells and their modulation by pro- and anti-inflammatory mediators would be useful for the design of novel therapies for OA treatment.

**Objectives:** To analyse the mediators released from co-cultures of OA chondrocytes and SF, and to elucidate the effect of Fn-fs and VIP on these cells.

**Methods:** Human articular chondrocytes (HAC) and SF from 4 OA patients were provided by the Rheumatology Service at Complejo Hospitalario Universitario A Coruña. Isolated cells were recovered and plated in SILAC DMEM-Flex lacking Arginine and Lysine. In the case of medium and heavy media, isotope-labeled Lysine and L-arginine were used. When 100% of labeling was reached, cells were put in co-culture and incubated in serum-free medium with or without Fn-fs (10<sup>-8</sup>M) or Fn-fs + VIP (10<sup>-8</sup>M) for 48h. Cell secretomes were separated on a 10% SDS PAGE gel. Gels were stained with Coomassie blue and the resulting lanes were desalted and concentrated via NuTip, subjected to liquid chromatography, using a Tempo nano LC equipped with a Sun Collect MALDI Spotter, and analyzed by MALDI-TOF/TOF. Identification of peptides and proteins and relative quantification were performed using Protein Pilot software (Sciex).

**Results:** Cell secretomes were analysed in 4 OA patients in duplicate. Database search (UniprotKB/Swissprot) and Venn's diagrams drawing tool (Venny 2.1.0) allowed us to identify 79 common proteins in the HACs-SF co-cultures. Among them, VIP was able to modulate 33 different proteins, significantly reducing 9 of them: CH3L1, PTX3, PGS2, MMP2, Complement C1R, Complement C3, TBA1, QSOX1, and CATB. These proteins include inflammatory and ECM proteins, proteases and complement system proteins among others, which play a main role in the OA pathogenesis.

**Conclusion:** VIP decreases inflammatory and degradative mediators in HAC-SF co-cultures, potentially slowing the progression of the disease and supporting its therapeutic role in osteoarthritis.

#### REFERENCES

- [1] Loeser RF, et al. Arthritis Rheum. 2012;64(6):1697-707.
- [2] Calamia V, et al. J Proteome Res. 2011;10(8):3701-11.
- [3] Pérez-García S, et al. Journal of molecular neuroscience : MN. 2014;52 (1):18-27.
- [4] Pérez-García S, et al. Am J Pathol. 2016;186(9):2449-61.
- [5] Pérez-García S, et al. J Cell Mol Med. 2016;20(4):678-87.
- [6] Zack MD, et al. Arthritis Rheum. 2006;54(9):2912-22.

Acknowledgement: This work has been supported by Instituto de Salud Carlos III, Spain, cofinanced by FEDER, European Union: RETICS program, RIER (RD16/0012/0002 and RD16/0012/0008), and the projects PI17/00027 and PI16/2124.

**Disclosure of Interests:** Selene Pérez García: None declared, Valentina Calamia: None declared, Tamara Hermida Gómez: None declared, Mar Carrión: None declared, Raúl Villanueva Romero: None declared, Yasmina Juarranz: None declared, Francisco J. Blanco Consultant for: AbbVie, Bioiberica, BMS, GSK, Grünenthal, Janssen, Lilly, Pfizer, Regeneron, Roche, Sanofi, TRB Chemedica, and UCB, Rosa P. Gomariz: None declared

DOI: 10.1136/annrheumdis-2019-eular.2764

AB0102

# GENERATION OF OSTEOARTHRITIC MESENCHYMAL STROMAL CELL LINES

<u>María Piñeiro-Ramil<sup>1,2</sup></u>, Rocío Castro-Viñuelas<sup>1,2</sup>, Clara Sanjurjo-Rodríguez<sup>1,2</sup>, Silvia Rodríguez-Fernández<sup>1,2</sup>, Tamara Hermida Gómez<sup>2</sup>, Francisco Javier de-Toro-Santos<sup>1,2</sup>, Francisco J. Blanco<sup>2</sup>, Isaac Fuentes-Boqueta<sup>1,2</sup>, Silvia Maria Díaz-Prado<sup>1,2</sup>. <sup>1</sup>Universidade da Coruña (UDC), A Coruña, Spain; <sup>2</sup>Instituto de Investigación Biomédica de A Coruña (INIBIC), A Coruña, Spain

Background: Bone-marrow mesenchymal stromal cells (MSCs) are multipotent self-renewal adult cells with high potential to regenerate the damaged tissues in degenerative diseases such as osteoarthritis (OA). Nevertheless, their usefulness for osteochondral Regenerative Medicine research is hampered by their proneness to senescence when in vitro cultured. Currently, MSC lines available are scarce and present limitations regarding their differentiation capacities. In addition, there is none OA MSC line available for research on this disease.

**Objectives:** The aim of this study was to generate and characterize immortalized human OA and non-OA MSC lines for their use in osteochondral Regenerative Medicine research.

**Methods:** For the generation of the immortalized MSC lines, SV40 large T antigen (SV40LT) and GFP-fused human telomerase reverse transcriptase (hTERT) were used. Primary MSCs derived from two hip OA patients and one hip fracture patient without OA were transduced by spinoculation at 800 xg for 45 minutes. Transgene expression was induced by valproic acid. Nuclear expression of SV40LT and GFP was tested by immunofluorescence. Proliferation and senescence were investigated through calculation of population doublings (PDs) at each passage after immortalization and  $\beta$ -galactosidase staining after 100 PDs for each MSC line. Maintenance of MSC characteristics in immortalized MSCs was tested by analysis of CD29, CD44, CD73, CD90, CD105, CD34 and CD45 expression by flow cytometry and cell differentiation experiments. Multi-lineage differentiation potential was analysed histochemical, immunohistochemical and molecularly.

**Results:** Three MSC lines have been generated: two OA and one non-OA. As shown by immunofluorescence, SV40LT is expressed in the nucleoplasm of these cells, while GFP-fused hTERT is expressed in the nucleoli. A constant proliferation rate thoroughout subculturing in addition to  $\beta$ -galactosidase negative staining confirms that immortalized MSC lines do not senesce, unlike primary MSCs. Expression of CD29, CD44, CD73 and CD90 and lack of CD34 and CD45 was conserved in immortalized MSC lines, while CD105 expression was altered for transduction status and passage. Both OA and non-OA immortalized MSC lines maintain their multipotency (namely, osteogenic, chondrogenic and adipogenic differentiation capacity).

**Conclusion:** Both OA and non-OA MSCs are susceptible to immortalization by SV40LT and hTERT. For they increased lifespan combined with keeping of most