Leukocyte-rich Platelet rich plasma (L-PRP). Platelets and leukocytes counts were performed on samples of blood, PRGF and L-PRP with a hematology analyzer. Morphological analysis of PRGF and L-PRP scaffolds was performed with scanning electron microscopy (SEM). Mechanical properties of the scaffolds were determined by a tensile test. PRGF and L-PRP scaffolds were incubated in the absence (non-inflammatory conditions) or presence of inflammatory condition. The conditioned media released by the different scaffolds was collected and the concentration of several growths factors and pro-inflammatory cytokines was determined by enzyme-linked immunosorbent assay (ELISA).

Results: Inclusion of the white blood cells in the PRP increased the presence of leukocytes from 0.2 to 19.3. The biological outcomes of the L-PRP under inflammatory conditions were dramatically altered compared with the outcomes showed by the PRGF. In fact, the structure of fibrin network was significantly modified due to the leukocytes and the presence of MMP-1 within the scaffolds increased from 2 to 79 ng/mL. As a consequence the leukocyte containing fibrin membranes were more fragile, reducing both their elongation capacity and the time until membrane rupture in more than 28% compared to PRGF scaffolds. The release of pro-inflammatory cytokines from scaffolds was significantly increased when leukocytes were included in the PRP and an inflammatory condition was evaluated. The amount of TNF-alpha, IL-6, IL-1 and IL-8 released from the L-PRP fibrin scaffolds was 31, 248, 151 and 381-fold higher than from PRGF scaffolds. The inflammatory response of fibroblast was significantly higher to L-PRP condicioned cultures than to PRGF ones.

Conclusions: Compared with PRGF, L-PRP induces significantly higher pro-inflammatory conditions. The inclusion of leukocytes alters fibrin network, reduces its biomechanical properties and increases the presence of pro-inflammatory cytokines such as TNF-alpha, IL-6, IL-1 and IL-8, which deeply influences the cellular inflammatory response.

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MOLECULAR RESPONSES OF THE INFRAPATELLAR FAT PAD MAY CONTRIBUTE TO PATHOLOGICAL CHANGES IN THE KNEE JOINT FOLLOWING IDEALIZED ANTERIOR CRUCIATE LIGAMENT SURGERY.

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Purpose: Severe injuries to the anterior cruciate ligament (ACL) require surgical reconstruction to restore mechanical stability in the knee joint. In spite of successful surgical reconstruction, in some cases the cartilage of the knee joint exhibits signs of degeneration analogous to symptoms related to osteoarthritis (OA). The causes leading to OA even after successful reconstruction remain to be defined. We developed a surgical model of an "idealized reconstruction of ACL" and hypothesized that immediate anatomical reconstruction of ACL will not lead to degeneration of the knee joint components. However, previous examination of synovium, cartilage and the ACL exhibited elevated molecular responses for inflammatory and degradative biomarkers early after the surgery, which subsided by 20 weeks. As the different components of the knee joint function as an integrated coordinated system, in the present study we examined the molecular responses in the infrapatellar fat pad (IPFP). The IPFP is present in the anterior compartment of the knee joint in close proximity to the synovial layers and cartilage surfaces. This suggests that the IPFP may be able to influence the catabolic and inflammatory processes in different components of the knee joint. Previous reports have observed pathological changes in this tissue either following arthroscopic procedures or secondary to knee joint insults such as patellar dislocation and tendonitis. This study was based on the hypothesis that following ACL-R, molecular changes will be initiated in the IPFP that will be sustained over time, thus acting as a molecular repository for fibrotic processes in the joint.

Methods: In this study, for analysis of IPFP tissue samples at 2 and 20 weeks, a total of 21 skeletally mature (3–4 year old) female Suffolk-cross sheep were allocated to 3 groups: a) 2 week ACL-R surgical group (N = 9), b) 20 week ACL-R surgical group (N = 7) and c) non-operated control group (N = 5). These tissues were assessed by real time q-PCR for mRNA levels of select molecules involved in remodelling and synthesis following surgery such as Collagen type I (Col-I), collagen Type III (Col-III) and associated growth factors such as transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF). The mRNA expression levels were normalized to 18S mRNA. ANOVA with Bonferroni post-hoc analysis was used to determined differences in expression levels between groups, using SPSS 19.0.

Results: Histological analysis of IPFP revealed a fibrogenic reaction of this tissue following ACL-R that was evident at 2 weeks post-injury. Molecular analysis revealed that the mRNA levels for TGF-β were elevated at 2 weeks following ACL-R surgery, but returned to levels close to the un-operated controls by 20 weeks. The state of the tissue correlates well with this molecular marker as it has been reported that TGF-β is a potent inducer of processes observed in fibrosis. VEGF is another growth factor that plays a major role in angiogenesis and was found to be elevated in the IPFP tissue in the early time points after ACL-R. Interestingly, elevations in Type I and Type III collagen were also observed in this fat pad, again consistent with a fibrogenic process in this tissue. The pattern of expression at the 2 and 20-week time points were similar to that of the growth factors discussed above.

Conclusion: The present study suggests that anabolic factors are activated and expressed in the IPFP tissue in a similar pattern as observed previously for synovium and cartilage in the same model. The initial fibrotic response did not resolve by 20 weeks post-injury, possibly compromising the function of the IPFP in the long term.

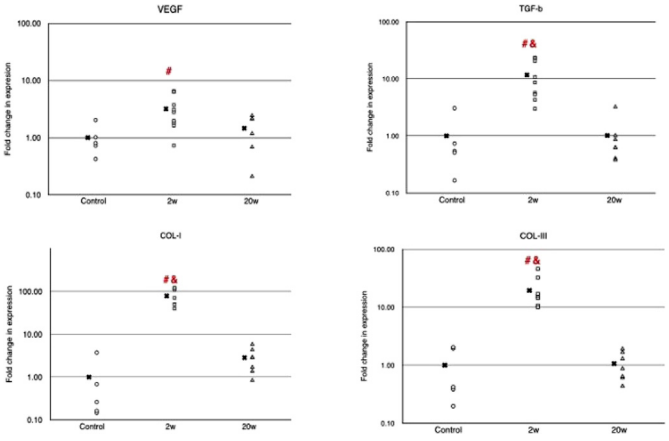

Fig. 1. Fold change expression levels of inflammatory mediators and adipokines measured infrapatellar fat pad after idealized ACL reconstruction in a sheep knee. Levels are normalized to 18S rRNA. Levels are normalized to 18S rRNA.

X – represents the moan expression level for each group and marker shown
○ □ Δ - represent a single measure from each sample in the group
- Significant difference between Control and 2w expression levels
& - Significant difference between 2 w and 20 weeks
@ - Significant difference between Control and 20 weeks

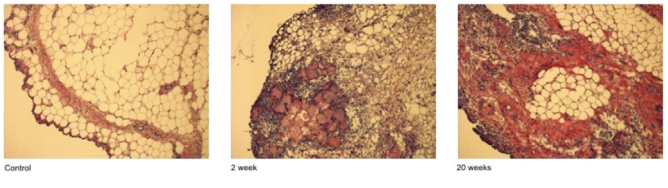

Fig. 2. – H&E images of infrapatellar fat pad after ACL reconstruction 10× magnification

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DIFFERENT DISTRIBUTION AND ACTIVATION DEGREE OF TH17 CELLS IN PERIPHERAL BLOOD IN PATIENTS WITH OSTEOARTHRITIS, RHEUMATOID ARTHRITIS AND HEALTHY DONORS: PRELIMINARY RESULTS OF THE MAGENTA CLICAO (CLINICAL CELL ANALYSIS IN OSTEOARTHRITIS) STUDY

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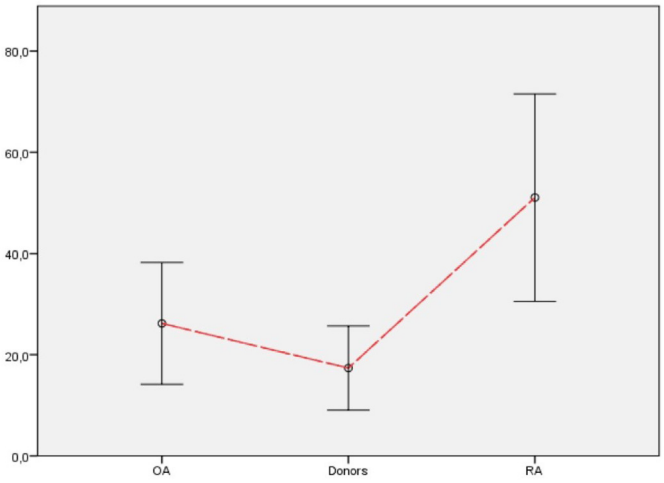
Background: Osteoarthritis (OA) as well as rheumatoid arthritis (RA) are chronic diseases associated with joint destruction and mobility impairment. Data about changes in immune system in RA and the significant

role of T cells, primarily Th17 cells, are now well recognised. On the other hand data about immunological profile in OA are limited, because OA has long been regarded in the past as a no inflammatory disease. Our study aim to measure the distribution and the activation degree of CD4+ Th17 in peripheral blood of OA, RA and age-matched healthy controls.
Methods: Patients with established diagnosis of RA according to ACR/EULAR 2010 criteria, knee or hip OA according to ACR criteria and volunteers healthy blood donors were eligible. Other inclusion criteria were a DAS28 between 3.2 and 5.1 or a WOMAC Likert score more than 50, respectively. Exclusion criteria were the presence of other autoimmune diseases, tumours or secondary osteoarthritis. Finally no changes in rheumatologic drugs were allowed from 3 months before enrolment. Multichannel flow cytometry was used for T cells subpopulation distinguishing and quantification using monoclonal antibodies against CD3, CD4, CD8, CCR6, CD38, CXCR3 and HLA DR. Participants were informed about the aim of the project and gave their written consent. The project was accepted by the Local Ethic Committee.
Results: We analysed blood samples of 91 subjects (75 females, 16 males). 15 Patients with well-defined RA, 56 with hip or knee OA and 20 healthy controls. Mean age was 45 ± 2.7 years old ($p > 0.05$ between groups). Blood samples from the RA patients had significantly higher count of CD4+CD38+DR+ (activated CD4 T cells) and Th17 (CCR6+CXCR3-) cells as compared to OA patients and control group (Figure) ($P < 0.01$). Furthermore the samples from the OA patients had an higher percentage of activated CD4 T cells and Th17 cells as compared to control group ($P < 0.05$)
Conclusion: According to the latest view of OA disease pathogenesis, our preliminary results give support to the hypothesis that OA may also (like RA) be a disease with an immunological/inflammatory involvement. In fact it seems that there is a quantitative but non qualitative difference in Th17 cells profile, including the expression of activation markers, between RA and OA.



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PERIODONTITIS DISEASE AS A MODEL OF INFLAMMATION AND BONE REMODELLING TO STUDY OSTEOARTHRITIS DRUG EFFECTS. RESULTS FROM A PILOT STUDY IN OA PATIENTS TREATED WITH CHONDROITIN SULFATE

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Purpose: Periodontitis is an inflammatory disease that affects 70% of the adult population. Its most important consequence is the loss of bone supporting the teeth. Its medical treatment so far has not shown to be effective so only different surgical techniques are useful in advanced stages. Osteoarthritis (OA) and periodontitis share physiopathological characteristics. OA patients also suffer from an important inflammatory component in the soft tissue and bone structural modifications. Because of the evolution of the disease, development of new treatments is difficult. Periodontitis could be a model for the study of OA, where the changes of tissues could be observed in a more directly way. Chondroitin sulfate (CS) has shown a positive effect in different chronic inflammatory diseases such as psoriasis, inflammatory bowel disease and OA. Periodontitis is the most prevalent inflammatory disease, but currently without specific treatment. New approaches for a safety and effective pharmacological treatments are required. The purpose of this study was to demonstrate that an effective drug in OA pathology may improve both the inflammatory symptoms and the bone resorption occurred in periodontal disease. This improvement would be reflected in changes in biochemical markers of inflammation and bone metabolism.
Methods: Observational, prospective, pilot study in 26 patients diagnosed with knee OA and periodontitis. Patients were treated with CS 800 mg/day(Condrosan®, Bioibérica SA) for 12 months. The Löe and Silness gingival index (used to assess soft tissue damage) and CPITN (Community Periodontal Index of Treatment Needs) index were evaluated at 0, 3, 6, 9 and 12 months with saliva collection. Orthopantomographys were performed at 0, 6 and 12 months to evaluate bone damage. Vertical lesions were measured using a periodontal probe at 0, 6 and 12 months. Different concentration of inflammatory markers (TNF -α, IL -1β, IL-18 and PGE_2) and bone metabolism (OPG, OPN, RANKL and MMP-8) were quantified in the saliva by ELISA or protein array. During the study, patients were asked to continue their usual oral hygiene without additional treatments. Statistical analysis was performed using Wilcoxon test for paired samples.
Results: Löe and Silness index decreased significantly after 6, 9 and 12 months of treatment ($p = 0.004$, 0.007 and 0.002 respectively). In contrast, no significant changes were observed in CPTIN values, probably due to the fact that only the most affected tooth is studied and it can differ between visits. Both orthopantomography as well as the vertical lesions evolution, showed significant improvement at 12 months ($p = 0.009$) and 6 and 12 months respectively ($p = 0.002$ and $p = 0.016$). For biochemical analysis results, patients with no gingival improvement were used as control group. The results show a better performance in the group of responders to treatment for inflammatory markers PGE_2, TNF-α and IL-1β , as well as bone metabolism markers, OPN, MMP -8, and the ratio OPG/RANKL. Responders groups PGE_2 levels showed a significant decreased after 12 months of treatment ($p = 0.033$), whereas IL-1β levels in non responding patients suffered a significant increased after 3 and 6 months of treatment ($p = 0.017$)
Conclusions: CS improves soft tissue inflammation after 6 months of treatment and bone support at 12 months in periodontal disease, similar to the already known effect of the drug in OA disease. This improvement is reflected in markers of inflammation and bone metabolism in saliva. Due to its efficacy and safety profile, CS is postulated as a good candidate for the treatment of this disease.

Intervertebral disc

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ADIPOKINES AND THE INTERVERTEBRAL DISC: DOES A BIOCHEMICAL LINK EXIST BETWEEN OBESITY AND BACK PAIN?

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Purpose: Obesity is a significant risk factor for development of low back pain and intervertebral disc (IVD) degeneration. The mechanism underlying this link is unclear but is commonly thought to arise from altered loading. However, adipokines such as leptin and adiponectin, produced by adipose tissue, are now known to be involved in degradative processes particularly in articular joints. We propose a similar link exists between these adipokines and intervertebral disc degeneration. Obese individuals are known to have higher concentrations of serum adipokines, there is increased local fat in chronic spinal conditions potentiating an increase in local adipokine levels and disc cells are reported to have leptin receptors and can synthesise leptin.
The aim of the study was to identify responses of nucleus pulposus (NP) and outer annulus fibrosus (OA) cells to leptin and adiponectin. Once identified, we proceeded to determine if synergistic effects exist in the presence of other pro-inflammatory cytokines.
Methods: Bovine intervertebral discs were used as a model system. Freshly isolated NP and OA cells embedded in 3D alginate beads, were