assessed by flow cytometry. Meniscus cells were co-cultured with progenitor cells in a 20:80 ratio in the absence of growth factors (n=2 donors, 3 pellets per donor). Glycosaminoglycan and DNA content was determined using a dimethylmethylene-Blue (DMMB) and PicoGreen assay.

Results: All progenitor and non-selected meniscus donors demonstrated osteogenic and adipogenic differentiation. All meniscus progenitors showed glycosaminoglycan deposition, indicating chondrogenic differentiation. In none of the of non-selected meniscus cells glycosaminoglycans could be detected by Safranin O staining (figure 1). Of the progenitor cells, 73-87% expressed the surface marker profile according to the ISCT MSC criteria(figure 2). Co-culture of meniscus cells with progenitor cells increased glycosaminoglycan deposition (figure 3).

Conclusions: Meniscus progenitor cells are present in the osteoarthritic human meniscus. In our experimental set-up, meniscus progenitor cells have trilineage potential with higher chondrogenic capacity than the total meniscus cell population. Flow cytometry indicates that meniscus progenitor cells express MSC markers. Due to the high chondrogenic potential, easy isolation and fast proliferation, meniscus progenitor cells are a promising cell source for regeneration of meniscus tissue.

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MACROSCOPIC AND HISTOLOGIC IMPROVEMENTS IN JOINT CARTILAGE, SUBCHONDRAL BONE AND SYNOVIAL MEMBRANE WITH GLYCOSAMINOGLYCANS AND NATIVE TYPE II COLLAGEN IN A RABBIT MODEL OF OSTEOARTHRITIS

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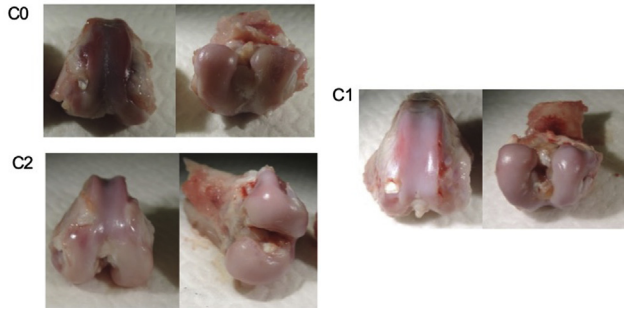
Purpose: To evaluate the effects of an oral combination of chondroitin sulfate (CS), glucosamine hydrochloride (Gl) and hyaluronic acid (HA), with or without native type II collagen (NC), on articular cartilage, subchondral bone and synovial membrane in an experimental model of osteoarthritis induced by anterior cruciate ligament section in rabbits.

Methods: This was a prospective, randomized, double-blinded experimental study. The study protocol was approved by an ethics committee in accordance with article 31 of RD53/2013. Fifty-four 10 weeks old female New Zealand white rabbits were distributed into 3 groups and received a daily oral administration of the following treatments: Group 0 (Control group - no treatment), Group 1 [CS (CS Bioactive®) + Gl + HA (Mobilee®)], and Group 2 [(CS + Gl + HA + NC (b-2Cool®)]. All active ingredients were provided by Bioiberica SAU, Barcelona, Spain. In each group, subjects were divided into 3 subgroups (n=6) based on survival times (28, 56 and 84 days). After quarantine period, section of the anterior cruciate ligament of the right stifle was performed in all rabbits under general anesthesia (intramuscular premedication: medetomidine 50μg/kg, fentanyl 10μg/kg, ketamine 5mg/kg and midazolam 0.5mg/kg; intravenous induction: propofol 2mg/kg; inhalatory maintenance: sevofluorane 2.6% and intravenous enrofloxacin 0.5mg/kg; intraoperative analgesia: fentanyl bolus 2μg/kg and CRI 1μg/kg/h; intravenous recovery: ranitidine 2mg/kg, metoclopramide 0.5mg/kg and meloxicam 0.4mg/kg). Afterwards, animals were kept under controlled conditions of temperature, humidity and photoperiod until sacrifice. After sacrifice, samples of lateral femoral condyle and synovial membrane were obtained. Macroscopic evaluation was performed following a cartilage surface scoring system described by Laverty et al. in 2010, and an osteophytosis staging semiquantitative scale described by Tsuromoto et al. in 2013. For histologic evaluation, and before observation under microscope, samples were fixed with 10% buffered formalin, decalcified and embedded in paraffine blocks to obtain longitudinal sections of 5 microns. Cartilage and subchondral bone sections from the femoral condyle were stained with hematoxylin-eosin and Masson trichrome stain, while synovial membrane sections were stained with hematoxylin-eosin. The OARSI semi-quantitative scale described by Laverty et al. in 2010 was used to evaluate matrix staining, cartilage structure, chondrocyte density and cluster formation. Changes in subchondral bone structure and synovial membrane cellular disposition were assessed by the semiquantitative scales described by Gerwin et al. in 2010. For the statistical analysis, a generalized lineal model has been used, with a statistical significance p-value of <0.05.



Results: As expected when using this model, all rabbits developed degenerative changes associated with osteoarthritis after anterior cruciate ligament section.When groups were compared, and overtime, macroscopic evaluation showed significantly lower values in Group 2, compared to groups 0 and 1, meaning that cartilage appearance in these rabbits was closer to that of a healthy one (Figure 1). Microscopically, the assessment of articular cartilage revealed significantly better results for groups 1 and 2, compared to Control, for matrix staining, cartilage structure, chondrocyte density and synovial membrane, indicating that these groups did not show the degree of degenerative changes observed in the Control group. Regarding microscopical evaluation of the subchondral bone, significantly better results were also seen in groups 1 and 2, compared to Control. On the other hand, histologic evaluation of the synovial membrane showed significantly lower values for Group 2, compared to groups 1 and 0; and significantly lower values for Group 1, compared to Group 0. Overall, the group in which joint structures showed values closer to those of a healthy joint was Group 2, followed by Group 1, and lastly by group 0, which featured a more severe degenerative process of osteoarthritis.

Conclusions: In a rabbit model of induced osteoarthritis, a beneficial treatment effect on articular cartilage, subchondral bone and synovial membrane was achieved following oral administration of the combinations CS+Gl+HA and CS+Gl+HA+NC. Moreover, the addition of NC to CS+Gl+HA allowed the combination CS+Gl+HA+NC to provide even significantly better results in terms of macroscopic cartilage evaluation and microscopic synovial membrane assessment. Although extrapolations between species should be made with caution, data presented herein supports the potential usefulness of these combinations in human and veterinary medicine for the multimodal management of patients with joint conditions.

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ARTICULAR CARTILAGE RESPONSE TO BLUNT VS SHARP LESIONS IN AN IN VIVO EQUINE CARPAL GROOVE MODEL

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Purpose: Chondral defects are common in humans and horses and may initiate the development of osteoarthritis. However, if, when and how they should be approached therapeutically is still subject of discussion. In the equine carpal joint, critical size of chondral lesions has been determined at 2mm diameter, beyond which no spontaneous healing occurs. However, the progression of degeneration is likely determined by other factors besides the size of a lesion. In bovine cartilage explants, sharp and blunt trauma lead to different responses in the tissue adjacent to the lesion. A better understanding of the progression of various forms of chondral lesions could help in determining the best (time of) intervention. Therefore, we investigated the long-term response of articular cartilage to artificially created blunt and sharp grooves in the equine carpal joint. We hypothesized that disruption of the collagen network of articular cartilage, combined with intensified loading would always lead to progressive degeneration, but that the presence of initial tissue loss (blunt grooves) would substantially accelerate this process.

Methods: In one randomly assigned front limb of nine adult female Shetland ponies the cartilage layers at the proximal surface of the intermediate carpal bone and at the radial facet of the third carpal bone