C8, C12, C15, C16, C18 linear alkyl amines and a benzyl amine, were tested for the inhibitory activity against *Ch*C. The inhibitor potency showed to be directly correlated to the length of the alkyl chain. The C16 alkyl amide derivative HYADD®4 was identified as lead compound (K_i= 6.2±1.6 μM), on the basis of the best compromise between potency and solubility.
With the aim of assessing the specificity of the effect, HYADD®4 was then screened *vs* 10 different human MMP catalytic subunits, showing higher selectivity for MMP8 and MMP13. The K_i against human MMP13 was found to be in the micromolar range (61.7 ± 7.0 μM).
Finally, since MMP13, unlike MMP8, is involved in OA progression, an *ex vivo* study was performed by incubating HYADD®4 in SF from patients with inflammatory arthritis: the K_i value obtained (106.1 ± 9.2 μM) was comparable to that observed in vitro, thus validating the inhibitory activity against MMP13.
Conclusions: These data suggest that the extremely hydrophobic side chain of HYADD®4, whose water solubility is boosted by the HA backbone, is pivotal in the mechanism of MMP competitive inhibition.
The polymer structure suggests that the alkyl side chain could easily and selectively dock the hydrophobic S_1' pocket in the MMP catalytic domain.
The hexadecyl derivative shows the best performance against MMP13 and MMP8, as confirmed also in an *ex-vivo* experiment in SF *vs* human MMP13. Furthermore, the intra-articular administration of the alkyl-modified HA as a treatment for OA, thanks to its site-selectivity and solubility, can overcome the common issues of the small molecule MMP inhibitors, such as systemic distribution and toxicity.

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EVALUATION OF A POLYACRYLAMIDE HYDROGEL IN THE TREATMENT OF INDUCED OSTEOARTHRITIS IN A GOAT MODEL: A RANDOMIZED CONTROLLED PILOT STUDY

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Purpose: Polyacrylamide hydrogel (PAAG) is an inert, non-degradable, non-immunogenic polymer gel with high viscoelasticity consisting of 97.5% sterile water and 2.5% cross-linked polyacrylamide. Its biocompatibility in soft tissues has been demonstrated. PAAG has recently been tested for the treatment of osteoarthritis (OA) in horses with highly encouraging results; however no standardized experimental studies have been done to explore its efficacy. The purpose of this study was to evaluate PAAG in the treatment of induced OA in a goat model.
Methods: A randomized controlled study was conducted involving goats with induced OA on the left stifle (knee) joint. OA was surgically induced by the transection of the medial collateral ligament, the bisection of the medial meniscus at its midpoint and partial-thickness incisions of the cartilage of the medial tibial plateau. Goats were allowed free exercise, and 3 months after surgery they were randomly divided into 2 groups: group 1 ($n = 4$): PAAG and group 2 ($n = 2$): saline solution (control). Treatments were injected intraarticularly. MRI of the left knee had been performed prior to surgery, at the time of injection (3 months) and 4, 5 and 7 months post-surgery. T1, T2/PD and Stir weighted MRI images were used to assess OA. All goats were clinically evaluated on ground and on treadmill and videotaped for evaluation by 3 blinded observers. Haematology, biochemistry and acute phase proteins were also assessed. The goats were euthanized 7 months after surgery, and gross pathology and histopathology, including immunohistochemistry for nerve endings ($n = 3$ joints), were performed on both femorotibial joints. The hardness of the joint capsule was measured in both groups using Instron ® 5564 testing system (HIS Global-Spec, MA, USA).
Results: At the end of the study, 75 % of the goats treated with PAAG were clinically sound, and 25 % of them had not improved, whereas the 2 control goats were still lame. In both groups, the values of haematology, biochemistry, or acute phase proteins were within normal range. MRI showed that in group one, 3 out of 4 goats had a decrease followed by a stabilization of OA lesions, while 1 goat had a mild progression of the OA lesions. In group 2, both goats had a mild or marked increase of OA lesions. Gross pathology inspection in group 1 demonstrated that all the operated knees showed typical signs of OA. The inner synovial lining was thickened, and the cartilage surface was uneven in all cases. The gel was seen in various amounts adhering to the inner side of the joint capsule in all the goats of group 1. Gross inspection of both goats in group 2 also showed cartilage lesions and synovial thickening, but the histopathological investigations revealed this to be more prominent in group 1 than in group 2. It comprised angiogenesis, collagen and synovial cell increase, and in the injected goats, also the gel. The nerve endings were normal looking and in normal numbers. The investigation of the joint capsule hardness showed that in the treated knee of the goats of group 1, the medial side (injected with PAAG) was always less hard than the lateral side.
Conclusions: This study demonstrated the efficacy of a novel treatment of OA, with 75 % of the goats treated with PAAG being clinically sound. Treatment with PAAG did not have any influence on haematology, biochemistry, or acute phase proteins. It induced a moderate synovial hyperplasia of the inner side of the capsule with trapped (integrated) gel, increased angiogenesis and collagen production. Preliminary pathology and joint capsule hardness data suggest that PAAG might act mainly on the joint soft tissue and especially the synovial membrane. PAAG might have 2 effects on OA joints: 1- Joint capsule was less hard on the treated (medial) than on the non-treated (lateral) side and had a lower hardness when compared to group 2. OA joints typically show joint stiffness - a major source of pain in OA. By decreasing the joint capsule hardness, and thus joint stiffness, PAAG might relieve the pain in the OA joint ("disease-modifying" effect). 2- MRI and pathology investigations have revealed a stabilization of OA lesions in the goats of group 1, which might be explained by the mechanical effect through the high viscosupplementation provided by PAAG that was still present in the joint cavity ("disease-stabilizing" effect). No adverse reaction was seen following intraarticular injection of PAAG. More investigations are needed to fully understand the mechanism of action of PAAG in improving clinical signs and in stabilizing OA. This pilot study may be used as a basis for further studies using larger animal numbers.

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EVALUATION OF NANOSTRUCTURED VECTORS FOR THE TREATMENT OF OSTEOARTICULAR PATHOLOGIES

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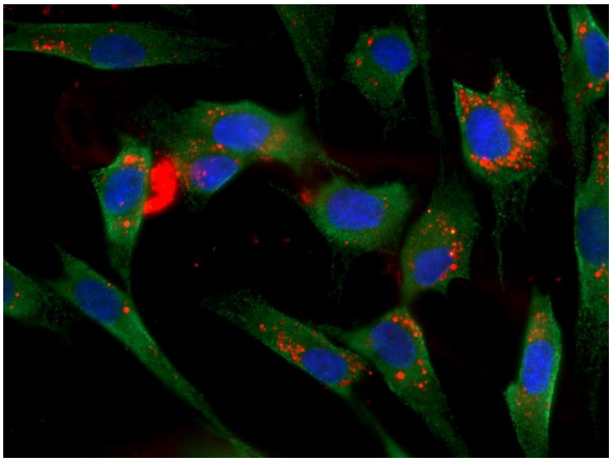
Purpose: One of the major problems in treatment of osteoarticular diseases is to reach cells inside the matrix to provide drug. Indeed, cartilage is an avascular tissue with a few cells feed by diffusion through a dense protein network (collagens, glycosaminoglycans). In this work we have designed polymeric nanoparticles (NPs) of poly (D, L-lactic/



glycolic acid)(PLGA) synthesized by a double emulsion method, which are biocompatible, biodegradable and can encapsulate water-soluble agents. Our NPs are labelled with BSA coupled to a fluorescent dye (Cyanine-3) to follow them by epifluorescent microscopy. As articular

cells expressed CD44, one receptor of hyaluronic acid (HA)(a main component of synovial fluid), the nanoparticles are recovered with HA in order to enhance targeting of cells. Here, we have studied the internalization kinetics of "empty" nanoparticles, and we have evaluated their neutrality on chondrocytes matrix synthesis, mesenchymal stem cell (MSC) differentiation and inflammatory response. Innocuity has been also evaluated in healthy animals, after direct intraarticular injection of labelled nanoparticles (inflammatory response, Extracellular matrix integrity).

Methods: Articular cells (chondrocytes, synoviocytes) and MSC are isolated from human donors, and cultured as primary culture. First, cells are exposed with 100 μg/mL of NPs from 2 to 12 hours. At the end of the kinetic immunofluorescence pictures with DAPI (nuclear staining) are realized to assess of the internalization of NPs, expression of inflammatory markers (IL1ß, TNFα and Cox2) are monitored by RT-qPCR analysis, and confirmed by PGE2 and nitrites measurement in supernatant.

In other hand we evaluate, with pellets culture system, the effect of NPs exposition on extracellular matrix synthesis by chondrocytes, with RT-qPCR analysis of specific markers (Col2, Aggrecan and COMP), and by histological study of pellets (Alcian blue staining of proteoglycans, Sirius Red staining of collagen). Finally by growing MSC into 3 different differentiation media, we investigate if NPs pre-treatment can interfere with differentiation ability of MSC onto chondrogenic, adipogenic or osteogenic pathway. RT-qPCR assays for differentiation markers according to culture conditions and specific staining of lipid vesicles or calcium deposits, allow us to confirm the differentiation of cells.

Intraarticular injections were realized in healthy rat's Knees. Structure of joint (synovium, cartilage, subchondral bone) was assessed by histological studies, performed at 7 and 10 days after injection (single and repeated).

Results: For the different cell types, NPs are found into cytoplasm after 6 hours of exposition.

Internalization of these NPs leads to an increase of inflammatory markers between 4 and 8 hours, basal level of expression being reached after 12 hours. Even if there is a weak increase of PGE2 and nitrites synthesis, that stay significantly lower than with LPS stimulation, our positive control of inflammation. NPs exposure, prior or after IL-1ß stimulation, does not aggravate the inflammatory response of these cells. When chondrocytes are exposed to NPs for 24 hours and then cultured in pellets for 28 days, there is no difference in matrix synthesis for the expression of mRNA and matrix deposition, as confirmed with histological exams. RT-qPCR and staining assays have also shown that despite a pretreatment with NPs, MSCs can be conducted onto adipogenic, osteogenic or chondrogenic differentiation pathways. Histological analyses of extracellular matrix integrity and inflammatory status do not demonstrate any differences for cartilage and subchondral bone structures but reveal a weak hyperplasia of synovial membrane, increasing with NPs concentration and the number of injection.

Conclusions: These NPs are rapidly internalized by human articular cells, with only moderated and transient pro-inflammatory effects. In addition there is no side effects on (1) ECM synthesis by chondrocytes or MSC and (2) differentiation process due to the presence of NPs. Labelled NPs with Cya3, once injected in joint of healthy rat, do not lead to an inflammatory reaction and/or modification of extracellular matrix integrity. NPs are mainly found in synovium and repeated injections do not generate severe adverse reaction. This drug delivery system can be used to deliver an active molecule into the knee joint, thanks to the absence of side effects.

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ROLE OF HIGH CONCENTRATIONS OF MANNITOL ON THE STABILITY OF HYALURONAN IN AN OXIDATIVE STRESS MODEL INDUCED BY XANTHINE/XANTHINE OXYDASE

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Purpose: Osteoarthritis(OA) is a degenerative joint disease associated with harmful action of reactive oxygen species (ROS). ROS are involved in the degradation of both aggrecan and high-molecular-weight hyaluronan (HMW-HA). The later plays a key-role in the joint lubrication and the visco-elastic and shock absorbing properties of the synovial fluid (SF). Viscosupplementation consists to inject intraarticularly exogenous HMW-HA to restore the SF rheological properties, that are dramatically decreased in OA. However the injected HA is also rapidly degradated by ROS, decreasing its effectiveness and duration of action.

The study objective was to evaluate the ability of Mannitol, a powerful oxygen free radical scavenger, to reduce HMW-HA ROS mediated degradation using a model of oxydative stress induced by xanthine (X) + xanthine oxidase (XOD). XOD is a flavoprotein that catalyzes oxidation of hypoxanthine to xanthine and then to uric acid generating high levels of superoxide anion.

Methods: Hyaluronan (MW# 0.8mDa) was submitted to an oxidative stress generated by the addition of X + XOD. Then solution of the same HA + 35g/L of Mannitol in PBS buffer was studied. Different enzyme concentrations were used and the HA properties were studied after 24 hours of contact at ambient temperature. Changes of the viscosity of the solution were assessed by rheometry with a rheometer at 25° C using a cone and plate geometry; steady-state viscosity was determined in Pa.s, as a function of the shear rate. HA MW was also determined by steric exclusion chromatography before and after oxydative stress.

Results: The presence of X/XOD degraded HA : 1) HA viscosity decreased as a function of XOD concentration, 2) HA MW decreased dramatically by 36.6%.On the opposite the presence of Mannitol stabilized HA : 1) HA viscosity remained stable, 2) HA MW decreased only slightly (-11.9%).

Conclusions: High concentrations (35g/L) of mannitol protect HA from ROS-mediated degradation. These in vitro data suggest that mannitol may increase the intra-articular residence time of HA and consequently may improve efficacy and/or effectiveness duration of viscosupplementation.

	Initial	+ 16μl XOD	+32 μL XOD
HA 0.8% MW	787 000 Da	621 450 Da	498 900 Da
Mannitol 3.5% + HA 0.8% MW	768 900 Da	717 750 Da	677 150 Da

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CHANGES OF THE BIOMARKERS IN SYNOVIAL FLUID AND CLINICAL EFFICACY OF INTRA-ARTICULAR INJECTION HYALURONIC ACID FOR PATIENTS WITH KNEE OSTEOARTHRITIS

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Purpose: Intra-articular injection hyaluronic acid (HA) for the treatment of patients with knee osteoarthritis (OA) has been commonly used in Japan. However, American Academy of Orthopaedic Sugerons (AAOS) announced intra-articular hyaluronic acid is no longer recommended in the 2013 guideline. Efficacy of the intra-articular HA still remain controversial. We have performed five weekly HA injections according to the Japanese insurance policy. Many biomarkers are reported: some biomarkers reflects cartilage metabolism and the others reflects inflammation reaction. We examined HA, chondroitin 4-sulfate (C4S), chondroitin 6-sulfate (C6S), keratan sulfate (KS) for cartilage metabolic biomarker and interleuikin-6 (IL-6) for inflammation biomarker. Il-6 is well known as inflammation cytokine in the patients with rheumatoid arthritis, but the change of Il-6 levels treated by the intra-articular HA in the patients with OA has not been reported. The purpose of this study was to investigate the change of the viscosity and biomarkers of synovial fluid and clinical symptoms before and after five weekly HA injections.

Methods: Fifty seven patients (61 knees) with symptomatic knee OA were enrolled between January 2010 and July 2013. We conducted 5

	1st	5th	Change	
SF volume (ml)	14.2	7.3	6.9	$P < 0.05$
HA (mg/ml)	1.50	1.72	0.22	$P < 0.05$
C4S (nmol/ml)	17.5	16.0	1.5	$P < 0.05$
C6s (nmol/ml)	59.7	50.2	9.5	$P < 0.05$
KS (μg/ml)	6.62	6.23	0.39	NS
IL-6 (pg/ml)	3733	457	3276	$P < 0.05$
Viscosity (mPa·S)	49.6	72.5	22.9	$P < 0.05$