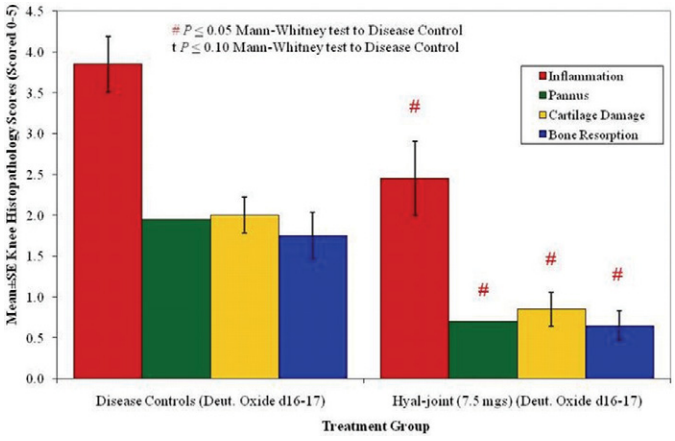

Figure 2

controls increased this rate to 0.61 μg/h, but Hyal-Joint® treated rats kept the value of normal rats (0.43 μg/h new HA).

Conclusions: Our results suggest that the use of Hyal-Joint® for the management of developing type II collagen arthritis in rats is safe and effective, having beneficial effects on the histopathology parameters in ankles and knees. Analysis of synovial fluid lavages indicates Hyal-Joint® reduces absolute synthesis rate of HA into synovial fluid of arthritic animals to normal levels.

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CHONDROPROTECTIVE ACTIVITY OF A FORMULATION THAT INCLUDES MANNOSAMINE

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Purpose: To evaluate the potential anti-arthritic activity of a formulation that includes mannosamine hydrochloride (Mann) and chondroitin sulphate (CS) in an *in vitro* chondrocyte model and in an animal model of osteoarthritis (OA) induced by meniscectomy.

Mann is an epimer of the natural Glucosamine Hydrochloride (Glu) and a constituent of bacterial cell walls and is involved in a range of metabolic processes. It is used as a probiotic in dietary supplements and functional foods (*Lactobacillus plantarum*, *Bacillus subtilis* and *Bacillus licheniformis*).

Methods: The chondroprotective activity of a formulation based on Mann + CS (60 mg/kg/day + 95 mg/kg/day) was tested using a guinea pig model of OA induced by partial medial meniscectomy. The following treatment groups were established: blank control group, operated control group, CS group (95 mg/kg/day), Mann groups (60 and 120 mg/kg/day) and CS + Mann (95 +60 mg/kg/day). The administration of the corresponding oral treatments (gastric gavage) was started the day after the surgery. The blank control and operated control group only received water for injection. The treatments were administered once a day for 70 days. After this period, the animals were sacrificed by anaesthetic overdose. The femorotibial joint of the right rear leg was removed. Histology was performed by a pathologist, in a blind manner, after staining with Hematoxilin-Eosin and Saffranin-O-Fast Green (or Toluidin Blue). The severity of the OA was evaluated by the modified histological/histochemical scale of Mankin et al (1971). The statistical evaluation between the different groups was carried out by means of the Mann-Whitney U test.

An *in vitro* study was performed using bovine chondrocytes cultured in alginate beads (n=4) in order to compare the activity of a mixture of Glu+CS versus Mann+CS on extracellular matrix degradation and apoptosis. Effects on apoptosis were determined by measuring Caspase-3/Caspase-7 activity after a three days of exposure to TNF-α. Effects on degradation were determined by measuring Metalloprotease activity (MMP activity) after stimulation with IL-1β for 48 hours followed by APMA activation. MMP activity was quantified using a fluorogenic MMP substrate.

Results: In the experimental conditions of the *in vivo* study, we found that there were no statistically significant differences between the operated group and the group treated with Mann. The group treated with CS showed improvement when compared to the operated group, but without statistical significance. In the group treated with the CS and Mann combination, statistically significant improvements were achieved when compared to the operated group ($p<0.05$).

The *in vitro* assays showed that Mann + CS leads to a statistically significant ($p<0.05$) reduction of apoptosis at medium and high concentrations and also a reduction in the MMP activity at low and high concentrations. These effects were not observed in the comparative mixture Glu + CS.

Conclusions: The efficacy study in guinea pig indicates that there is a synergic effect between Mann and CS when they are administered together. The *in vitro* studies suggest that the combination Mannosamine Hydrochloride and Chondroitin sulphate may have a higher chondroprotective activity than the already marketed formulation of Glucosamine Hydrochloride and Chondroitin Sulphate.

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A COMBINATION OF A SPECIFIC COLLAGEN HYDROLYSATE WITH A REFINED ROSE HIP EXTRACT HAS A POSITIVE EFFECT ON THE EXTRA CELLULAR MATRIX MAINTENANCE OF CARTILAGE CELLS

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Purpose: Experimental studies have demonstrated a stimulatory impact of collagen hydrolysate on the biosynthesis of cartilage tissue. In addition, rose hip extracts are described for an anti-inflammatory potential.

Aim of the present study was to investigate the influence of a combination of both collagen hydrolysate and a rose hip extract in order to determine the stimulatory and anti-inflammatory efficacy on the matrix synthesis of articular cartilage cells.

Methods: For the investigations a combination of a specific collagen hydrolysate (Fortigel™, GELITA AG, Germany) and a processed aqueous rose hip extract (nutrifin™ motion, Finzelberg, Germany) was used.

Mature articular chondrocytes were isolated from cartilage tissue and the cells were cultivated in cell culture medium at 37°C under oxygen reduced conditions (5% O_2).

Three days after cell preparation chondrocytes were treated with a combination of 0.5 mg/ml FORTIGEL™ and 0.05 mg/ml nutrifin™ motion.

At the end of the observation period the collagen and proteoglycan biosynthesis of the chondrocytes was quantified and the results were compared to the corresponding data of the untreated control cells. To confirm the stimulatory effect on matrix macromolecule synthesis mRNA expression of aggrecan and type II collagen was determined.

Moreover, the inhibitory effect of the FORTIGEL™/nutrifin™ motion combination on inflammatory cytokines (TNFα, IL-6) and matrix degradation (MMP-1, MMP-13) were tested.

Results: The supplementation of a FORTIGEL™/nutrifin™ motion combination led to a statistically significant ($p<0.05$) stimulation of the extra cellular matrix synthesis of articular chondrocytes. Type II collagen and aggrecan biosynthesis was increased by more than 45% in comparison to the untreated control cells.

Moreover, an anti-inflammatory effect as well as an inhibitory impact on MMP-1 and MMP-13 expression could be observed after FORTIGEL™ and nutrifin™ motion administration compared to the untreated chondrocytes.

Conclusions: The results indicate the efficacy of FORTIGEL™ and nutrifin™ motion to stimulate extra cellular matrix biosynthesis and to decrease inflammatory and catabolic processes. These data suggested that especially a combination of both substances seems to be of particular therapeutic relevance in the treatment and prevention of osteoarthritis.

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EVALUATION OF THE ADHESION TO THE EULAR AND OARSI RECOMMENDATIONS FOR THE TREATMENT OF KNEE AND HIP OSTEOARTHRITIS IN CURRENT PRACTICE

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Purpose: The aim of this study was to evaluate, in real life, the acceptance of the EULAR and OARSI recommendations for the treatment of knee and hip osteoarthritis (OA) in the two districts of the Neuchâtel Mountains.

Methods: In March 2008, a questionnaire was sent to all the general practitioners (GP = 23), internists (IM = 22), orthopedic surgeons (ORTHO = 8) and rheumatologists (RHEUMATO = 3) of the districts of Le Locle

and La Chaux-de-Fonds (population of 55'000 people). The anonymous questionnaire, presenting the 10 EULAR and 25 OARSI recommendations for the treatment of hip and knee OA, asked for some demographic data of the physicians and for the knowledge and acceptance of the presented recommendations.
Results: They were expressed as a "strength of acceptance" (SoA), based on a semi-quantitative evaluation (1 to 5/5) and expressed in percent for comparison to the "strength of recommendation" (SoR) reported by EULAR and OARSI.Twenty-three questionnaires (41%) were returned. Seven of the ten GP, one of the six IM, one of the four ORTHO who responded and the three RHEUMATO knew and applied some of the EULAR recommendations. Only one GP, one ORTHO and one RHEUMATO knew the OARSI recommendations. The SoA was a little less for the primary care physicians (GP + IM) than for the specialists in the musculoskeletal diseases (ORTHO + RHEUMATO) for the EULAR (85 versus 89%) and for the OARSI (73 versus 78%) recommendations. None of the EULAR or OARSI recommendations gained a full SoA by the primary care physicians although two EULAR and one OARSI recommendations obtained a complete SoA by the specialists in the musculoskeletal diseases.
Conclusions: In this sample of physicians, most IM and ORTHO ignored the EULAR and OARSI recommendations for the treatment of hip or knee OA. Nonetheless, a majority of primary care physicians and specialists in the musculoskeletal diseases adhered to the most of them with occasionally a greater SoA than the proposed SoR. An effort for a better diffusion of these recommendations is justified among IM and ORTHO.

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OFLOXACIN LOADED, IN - SITU - GELLING, CALCIUM ALGINATE HYDROGEL IN THE LOCAL TREATMENT OF BONE AND SOFT TISSUE INFECTIONS IN ORTHOPAEDIC SURGERY

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Purpose: Infection comprises a potentially serious complication following joint replacement surgery as a result of osteoarthtritis, and also poses an even greater challenge to the health care in view of the number of severe bone, joint and soft tissue suppurations accompanying high-energy injuries.
The treatment of these infections is based on appropriately radical surgical debridement and long - lasting specified parenteral antibiotic therapy. The material most commonly used for local antibiotic therapy is a bone cement, compounded from a liquid monomer and a mixture of solid methyl and methacrylate PMMA components. As polymerization proceeds in the patient as an endothermic reaction at a temperature sometimes exceeding 100°C, the added antibiotics should be heat-resistant and must not influence the mechanical characteristics of the PMMA.
The aim of our research work was to develop an injectable drug - delivery system that forms an elastic gel within a short time after its injection into joints, bone cavities or subcutaneous tissues, prevented from flowing out by virtue of its high viscosity, while the drug release is controlled by the biodegradable polymer network.
Methods: Sodium alginate was chosen as a natural polymer which has been widely investigated for drug delivery. It also has the potential for use as a scaffolding material for tissue engineering because of its structural similarity to the natural extracellular matrix, its gentle gelling kinetics, and its low toxicity when purified.
Ofloxacin, selected as active agent at a concentration of 3%, is a synthetic broad-spectrum antimicrobial agent for oral and intravenous administration, with an in vitro activity against a broad spectrum of Gram-positive and Gram-negative aerobic and anaerobic bacteria. Crosslinking of the viscous - flowing sodium alginate mucilage was carried out ex tempore with calcium-sulphate in the presence of ethylenediaminetetraacetate (EDTA), using two injection syringes joined by a thin tube. Rheological measurements to find the suitable hydrogel delivery systems were carried out with a Paar Physica MCR 101 rheometer. An in vitro Franz vertical cell diffusion drug release test and in vitro microbiological evaluation of the drug release via the Kirby-Bauer disk diffusion method were performed.
Results: As a result of crosslinking, a marked increase in viscosity was observed, which proved suitable for our application purposes. Release from the more viscous hydrogel containing 3% sodium alginate was more delayed than that from the 2% sodium alginate - containing gel. However, considering the effect of water content on the diameter of inhibition zones it can be concluded, that the higher viscosity led to the formation of

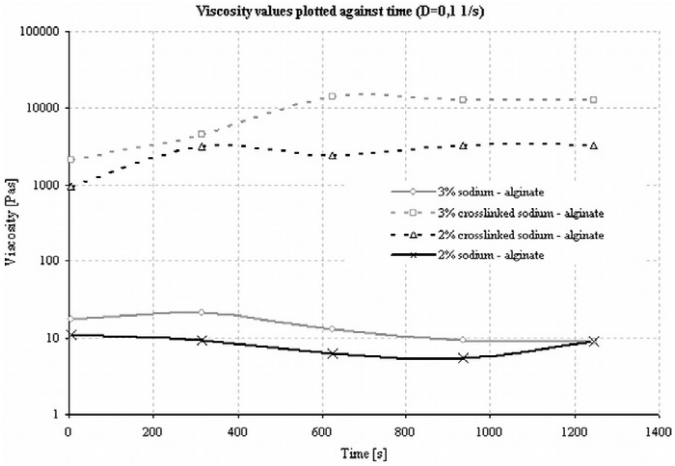

Figure 1

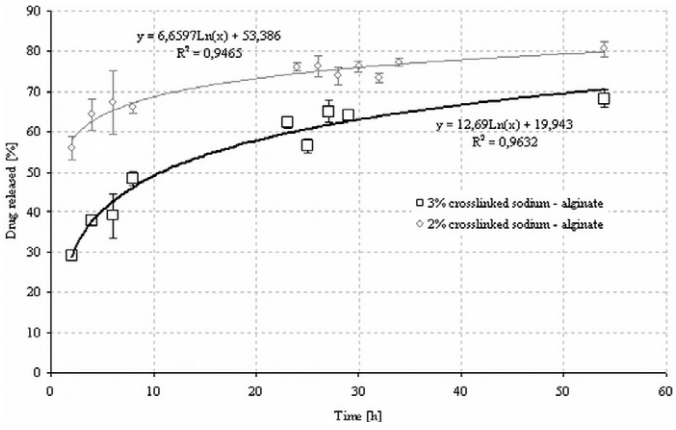

Figure 2

wider inhibition zones. Microbiological evaluation of the antibiotic release disclosed that antibiotic was released from the discs in all cases.
Conclusions: Ofloxacin released effectively from the crosslinked calcium alginate, and increased the inhibitory zones around the hydrogel discs. It seemed to be effective against most pathogens with a resistance to other antibiotics, emerging in our orthopaedic clinical practice in recent years. The present results suggest that such crosslinked calcium alginate hydrogels are good candidates for replacement of the currently used non-biodegradable bone cements with high polymerization temperature in certain orthopedic surgical interventions.

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ANTI-INFLAMMATORY ACTIVITY AND ABSORPTION OF A NATURAL ROOSTER COMB EXTRACT (HYAL-JOINT®)

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Purpose: To evaluate the potential anti-inflammatory activity *in vitro*, as well as the absorption of a natural extract from rooster comb (Hyal-Joint®).
Methods: Two *in vitro* assays were performed to determine the potential activity of Hyal-Joint®. The effect on inflammation was determined by using human dermal fibroblasts stimulated with IL-1β and co-treated with 3 concentrations of the nutraceutical extract (5, 50 and 500 μg/ml). Levels of Prostaglandin E_2 (PGE_2) and Metalloprotease-1 (MMP-1) were determined by EIA. The compound NS 398 at 1 μM was used as a positive control of cyclooxygenase-2 (Cox-2) inhibition in this assay.
The absorption of the extract was determined using the everted gut sac model in rats. Male OFA-strain rats weighing around 200g were sacrificed by cervical dislocation and segments of 4cm of jejunum, duodenum and ileum were removed, rinsed with Krebs-Henseleit solution and everted.