occurred at a rate of 60%, 63%, 67%, 70%, and 90% in AL100, AL20, AL500, HA, and NS, respectively.
Histology. In the NS group, loss of the superficial layer, fibrillation, and cleft were observed. In the HA and the AL500 groups, cartilage degeneration including fibrillation, fissures, and loss of proteoglycan was observed in the femoral condyle. An obvious reduction in the severity was found in the AL100 group. The overall degenerative score of the AL100 group tended to be the lowest, indicating that AL100 suppressed degradation. The overall scores of the treatment groups were significantly lower than that of the NS group (AL20, AL100, and AL500 vs NS, $p<0.01$; HA vs NS, $p<0.05$).
Friction. The friction coefficients of the AL100 and the AL20 group were significantly lower than that of the NS and the HA group ($P<0.05$).

Table 1. Histological scores and friction coefficient

	Histological score	Friction coefficient
NS	21.3±1.3	0.0294±0.0014
HA	17.3±1.2	0.0235±0.0013
AL20	14.9±0.9	0.0148±0.0027
AL100	12.6±0.9	0.0122±0.0037
AL500	15.4±0.9	0.0232±0.0117

Conclusions: The current study is the first to examine the influence of alginate materials on OA progression in vivo. Our findings suggest that intraarticular administration of the UPLE-alginates is effective in preventing articular cartilage degeneration and on improving joint lubrication of OA knees induced by ACLT. In terms of molecular weight dependency, AL100 (1.0×10^6 Da) has more therapeutic effects on OA progression. Based on these results, we reasonably conclude that the UPLE-alginates, especially AL100, have promising potential for becoming an effective agent of intraarticular injection for preventing OA progression.

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EFFECTS OF INTRA-ARTICULAR INJECTIONS OF HYLAN GF-20 ON SERUM AND URINE BIOMARKERS IN PATIENTS WITH KNEE OSTEOARTHRITIS: THE BIOVISCO STUDY

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Purpose: Viscosupplementation (VS) by intra articular (IA) injection of hyaluronic acid (HA) is widely used to reduce pain in patients with knee osteoarthritis (K-OA). However little is known on its effect on joint metabolism as well as on its possible structure modifying effect. Objectives: To investigate the effect of VS on circulating OA biomarkers in patients with K-OA.
Methods: Prospective open label study. 51 patients with unilateral symptomatic K-OA (ACR criteria; Kellgren-Lawrence grade I to IV) received an IA injection of 2mL of HA (hylan GF-20) IA injection on days (D) 1, 7, 14 and were followed 3 months. At D-15 patients were examined and X-rays were performed, in order to exclude patients with bilateral K-OA, or those with more than 3 OA joints including the target knee. From D-15 to D90 concomitant therapies were unchanged. Walking pain (WP) on VAS was obtained at each visit. Clinical response was defined as a WP decrease >30 mm between D1 and D90 (50% improvement). Urine (U) and serum (S) samples were obtained, using a standardized procedure, 2 weeks before the first injection (D-15), then at D1 (1st injection), D30 and D90. S-C2C, S-Cartilage oligomeric protein (S-COMP), S-HA, S-CS846 epitope, S-type II collagen propeptide (S-PIICP) and U-type II collagen C telopeptide (CTX II/creatinin) were assayed. Variations over time for each biomarker were studied using Wilcoxon rank sum test.
Results: 45 patients (mean age 57.7, mean BMI 26.7) were analyzed. At baseline there was no difference between ITT and per-protocol population. Between D-15 and D1 there was no significant difference for any biomarkers (all $p>0.05$), indicating a good reproducibility in S and U measurements and the absence of spontaneous variation over time. At D1 WP was correlated with U-CTX II/creat (p=0.006). Between D1 and D90: Mean (SD) WP decreased from 57.7 (15.4) to 29.3(22.9) mm ($p<0.0001$). No variation was found for any S-biomarker. By contrast U-CTX II/creat was reduced by 20.5% and decreased significantly between D1 and D90 (385.1 vs 306.0 ng/mmol creat; p= 0.02). Furthermore U-CTX II and S-HA levels at baseline were both but independently predictive of clinical response to treatment (p=0.03 and p=0.02) even after adjustment for age, gender and BMI. Further more in logistic regression including age, BMI, bilaterality, KL grade, OA at other joints, DMOADS consumption and U-CTX II/creat at baseline, there was a significant correlation between clinical response and U-CTX II/creat level variation (p=0.03).
Conclusions: This study suggests that hylan IA injections are able to modify the knee joint metabolism in patients with OA resulting in a decrease in urine CTX II concentrations, particularly in patients with the highest levels of U-CTX II and HA before treatment. Further studies coupling biomarkers and imaging techniques are needed to investigate the possible chondroprotective effect of hylan in K-OA.

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CHARACTERIZATION OF SUSTAINED RELEASE NATIVE AND MODIFIED HUMAN SFLT01 FORMULATION FOR INTRAARTICULAR DELIVERY TO TREAT OA PAIN

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Purpose: VEGF is both a potent angiogenic and vascular permeability factor that is crucial in endochondral ossification involved in bone formation and remodeling. We hypothesize that osteoarthritis (OA) pain-associated bone marrow lesions, effusion, and synovitis are in part, driven by VEGF mediated increases in vascular permeability. Soluble fms-link tyrosine kinase 1 (sFlt1), a variant of VEGF receptor Flt1, can potentially block the function of VEGF. Our previous work demonstrated that VEGF inhibition using virally delivered sFlt decreased synovitis and pain marker expression in a rabbit OA model. However, the biological joint half life of recombinant human sFlt01, a VEGF neutralizing Fc fusion protein, was demonstrated to be just a few hours. In order to prolong the joint half life of sFlt01, we constructed a modified delivery form of this protein, verified its bioactivity, and confirmed enhanced stability compared to sFlt01 in accelerated stability tests up to 3 months.
Methods: Modified sFlt delivery construct TCEP reduction and CuCl2 oxidation were used to create a dimerized modified sFlt delivery construct, which was subsequently column purified and compared to native sFlt as well as the monomer forms of the modified delivery construct on silver stained SDS PAGE gels.
In vitro VEGF ELISA binding assay A two-fold serial dilution of sFlt01 or the modified sFlt delivery construct starting from 10,000 pM was made in assay medium (M199 media + 5% FBS + 1x Penn/Strep). VEGF165 (10 pM) was then incubated with the serial dilutions. Unbound VEGF for each dilution was measured by ELISA to identify whether any shift had occurred between VEGF neutralization by the modified sFlt delivery construct vs. native sFlt01 control.
Accelerated stability test Modified sFlt delivery construct (0.25 mg/ml) was incubated in PBS with 1X HALT protease inhibitor cocktail with EDTA (Pierce) at 45°C and aliquots drawn and flash frozen at -80°C. Unmodified sFlt01 at the same concentration was used as a control. The samples were collected over different time points in both 36 and 94 day stability tests. The samples with or without PNGase F treatment was further analyzed by silver-stained reducing SDS PAGE.
Results: TCEP reduction and CuCl2 oxidation resulted in successful dimerization of the modified sFlt delivery construct.
TCEP reduced protein monomer showed ~35 fold higher EC50 vs. native sFlt01 whereas Cu2+ oxidized dimer showed 2.2 - fold high EC50 compared to sFlt01.
From the accelerated stability test data, sFlt01 control showed increase amount of ~35 kDa product as well as high molecular weight of ~115 kDa product in silver stained reducing SDS PAGE without PNGase treatment. The modified sFlt delivery construct showed greater stability at elevated temperature, generating less high molecular weight aggregate and fragmentation compared to sFlt01 control at both 36 and 96 days.
Conclusion: The generated modified sFlt delivery construct expressed from HEK293 can be efficiently dimerized.
Copper-generated protein dimer retains high affinity for VEGF binding, comparable to native sFlt01.
The modified sFlt delivery construct appears more stable at elevated tem-

perature vs. sFlt01, suggesting a sustained release formulation is possible without fragmentation.

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ACCURACY OF INTRA-ARTICULAR INJECTIONS IN THE KNEE: A SYSTEMATIC REVIEW

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Purpose: Intra-articular injections in the knee joint are commonly used for therapeutic and diagnostic goals concerning knee pathology. Several approaches are used to establish an intra-articular injection in the knee joint, however accuracy rates differ per approach. The primary objective was to summarize the evidence concerning the accuracy of different approaches for intra-articular injections in the knee.
Methods: The literature was systemically reviewed in online databases Pubmed and Embase until June 2009. Two reviewers (JH, MR) independently applied the inclusion and exclusion criteria and inclusion was reached by consensus. Risk of bias of the included studies was assessed independently by 2 reviewers using the QUADAS-tool. Study characteristics, accuracy data, other outcome measures, results and conclusions were independently extracted by 2 reviewers. A trained statistician pooled the accuracy rates per used injection approach.
Results: In total, 9 studies were included. The superolateral approach with the knee in extension was studied most (230 injections) and resulted in the highest pooled accuracy of 89% (95% C.I. 85%-93%). Pooling of the medial midpatellar approach, the anterolateral approach and the anteromedial approach resulted in the lowest pooled accuracy rates, respectively in 56% (95% C.I. 46%-68%), 70% (95% C.I. 64%-77%) and 71% (95% C.I. 65%-78%).
Conclusion: Based on the results of this systematic review the authors recommend the superolateral approach with the knee in extension for the intra-articular injection of the knee joint.

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CLINICAL RESULTS OF OPEN WEDGE HIGH TIBIAL OSTEOTOMY FOR OSTEOARTHRITIS AND OSTEONECROSIS OF THE KNEE

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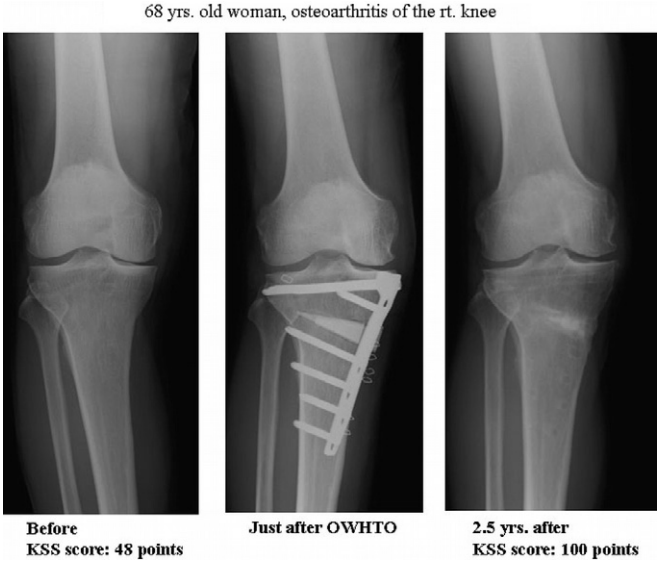
Purpose: We performed clinical and radiographic evaluation of patients with medial compartmental osteoarthritis and spontaneous osteonecrosis of the knee who had undergone treatment with opening-wedge high tibial osteotomy (OWHTO) followed by early full weight bearing. OWHTO procedure were performed using TomoFix™ and bone substitute materials.
Methods: OWHTO was performed in 93 knees of 77 patients of an average age of 68 years (range 52-82) at the time of the operation. Sixty-three knees from 47 patients were diagnosed as primary osteoarthritis (OA) and a further 30 patients were diagnosed as spontaneous osteonecrosis (ON). We established an early weight bearing program during which these patients were permitted partial weight bearing exercise one week after their osteotomy. Patients who performed OWHTO for one side started full weight bearing walk at two weeks, and patients simultaneous bilateral cases (11 patients) started full weight bearing at three weeks post-surgery. The average follow-up period was 49 months (range 24 to 83 months). Clinical examinations of the knee joints in our patient cohort consisted of both subjective and objective parameters that were recorded and documented using the American Knee Society Knee Score (KSS) and Function Score. These evaluations were carried out presurgically and at the time of follow-up. Additional clinical findings that were assessed included range of motion, Japanese-style sitting, and possible post-surgical complications. Radiological evaluations were carried out on the femoro-tibial angle (FTA), using an AP weight bearing radiograph of a single leg, with the knee joint in extension. A weight bearing line (WBL) ratio was calculated using standing long-cassette radiographs of the lower extremities.
Results: Functional assays, including the American Knee Society Score and Function Score, showed significant improvement from 49±11 to 91±7.7 points, and 62±13 to 95±8.2 points, respectively. Prior to surgery, the average femoro-tibial angle during standing was 181±2.5° (1° anatomical varus) but measured 169±2.2° (11° valgus) at the time of follow-up. There



were no instances of non-union or implant failure in any of our patient subjects. Furthermore, 54 of the patients in our study group (70%) could sit comfortably in the Japanese style after surgery. Their average FTA was 181.3±2.4°(11° varus) and average WBL ratio was 17.2±16.5%, indicating that the WBL had shifted toward the medial compartment. After surgery, the FTA improved to 169.6±2.3° (10° valgus) and the WBL ratio shifted to 62.9±12.5%, indicating that the WBL had moved toward the lateral compartment of the knee.
Conclusions: We demonstrate that an early weight bearing exercise program enables full weight bearing at two weeks after OWHTO with TomoFix and artificial bone wedges. Overall, this combination was a highly successfully course of treatment for correcting knee malalignment in patients with medial compartmental OA and ON of the knee.

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EFFICACY AND SAFETY OF THREE INTRA-ARTICULAR INJECTIONS WITH JOINTEX MINI FOR THE TREATMENT OF SYMPTOMATIC CARPO-METACARPAL JOINT OSTEOARTHRITIS

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Purpose: To investigate the efficacy and safety of a three intra-articular injection course with Jointex Mini (Hyaluronic Acid 8mg/1ml, Chiesi Farmaceutici S.p.A., Parma, Italy) for the treatment of symptomatic OA of the CMC.
Methods: Fourty-eight female patients affected by symptomatic CMC OA (age 51-90 years; 67±9 years, mean ± SD) were treated with three once-weekly intra-articular injections of Jointex Mini. All subjects met ACR criteria for hand OA and had CMC OA grade 1-4 according to Kellgren and Lawrence on standard X-ray performed within 6 months before the inclusion. Twelve patients showed bilateral OA so that globally 60 CMC joints were treated. Patients were followed for
a 3-month period after the last injection. Treatment efficacy was assessed through visual analogue scale (VAS) pain quantification (baseline; 2nd and 3rd injection; one and three months after the last injection). Side effects were recorded.
Results: VAS was significantly reduced after the first injection (2nd injection vs baseline, $p<0.005$; 3rd injection vs baseline, $p<0.0001$; 3rd injection vs 2nd injection, $p<0.05$) and reached the slowest score one month after the last injection. The efficacy was maintained for all the 3-month follow-up period (one month vs baseline, $p<0.0001$; three months vs baseline, $p<0.0001$; - one month vs 3rd injection, p=n.s.; three months vs 3rd injection, p=n.s.). Only minor side effects were observed (mild pain and/or ecchymosis in injection site).
Conclusions: Our study supports viscosupplementation with Jointex Mini as a safe and efficacious approach for symptomatic CMC OA. Our schedule based on three weekly intra-articular injections supplies pain relief lasting as long as 3 months with negligible side-effects.