Methods: 24 skeletally mature New Zealand White rabbits underwent anterior cruciate ligament transection. 6 additional sham operated animals were used as controls. 10 days after the surgery the transected animals received an intraarticular injection of CD-RAP encapsulated in liposomes or liposomes alone as vehicle control. Three different dose levels of CD-RAP were tested. Thereafter the animals were injected every 10 days until the end of the 9-week treatment period. The animals were analysed macroscopically, radiologically, and histologically. To score the radiological and histological data, the Kellgren and a modified Mankin score, respectively, were used.

Results: CD-RAP treatment resulted in reduced osteophyte formation and reduced joint space narrowing. This effect was significant as shown by the radiological scoring. There was a trend towards middle and high dose CD-RAP being more efficacious than the low dose group but this was not statistically significant. The histological evaluation by the modified Mankin score, which evaluated cartilage structure, cells, matrix (safranin-O), and tidemark integrity, confirmed the radiological results. The average scores for sham, vehicle, and treatments groups (low, mid and high dose CD-RAP) were 1.12±1.19, 7.28±2.19, 7.22±2.69, 4.08±0.8, 4.47±1.93. Both middle and high dose CD-RAP groups showed a significant improvement over the vehicle group. Looking at the individual parameters, CD-RAP appeared to impact structural integrity as well as matrix content.

Conclusions: These data suggest that treatment with the cartilage specific protein CD-RAP may be a new therapeutic option for osteoarthritis patients. Next steps will include the long-term evaluation of liposome-encapsulated CD-RAP in a large animal model.

A36
MODULATION OF COLLAGEN NETWORK FORMATION TO IMPROVE FUNCTIONAL PROPERTIES IN CARTILAGE REGENERATION

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Purpose: Osteoarthritis is characterized by the progressive destruction of articular cartilage. Damage to the collagen network is often considered irreversible, whereas proteoglycan loss is reversible. Understanding mechanisms of repair of the collagen network is therefore key for functional repair of cartilage. The present study was designed to test whether collagen synthesis and subsequent crosslinking could be influenced such that a functional collagen network is formed.

Methods: Bovine articular chondrocytes were cultured in alginate beads for 5 weeks in the presence or absence of the lysyl oxidase inhibitor BAPN (0.25 mM) followed by 5 weeks without BAPN (+/− or −/−). Collagen deposition and amount of crosslinks (HPLC) and gene expression of collagen type 1 and 2 and SOX9 (RT-PCR) were measured. Susceptibility of the extracellular matrix to in vitro degradation by MMP-1 was examined after 70 days of culture by overnight incubating 4 alginate beads with 100nM MMP-1. Alginate constructs of 3 mm thick and 6 mm in diameter were used to determine the equilibrium modulus and de secant modulus by unconfined compression. During cartilage tissue regeneration an effect on the functional properties of the construct.

Results: Complete inhibition of collagen crosslinking for 5 weeks by BAPN increased the collagen deposition 1.8 times, with a concomitant increase in tissue stiffness (the equilibrium modulus increased from 451 Pa to 879 Pa), despite the absence of crosslinks in the BAPN condition. When after 10 weeks the amount of collagen is further increased, crosslinks also seem to be important in improving the stiffness of the matrix. From week 8 onwards, i.e. 3 weeks after removal of the crosslink inhibitor, the crosslinks returned approaching the level of the control condition at week 10. With equal amount of crosslinks (−/+ and −/−) the equilibrium modulus is higher when more collagen was present. However, the total absence of collagen crosslinks together with more collagen resulted in a lower secant modulus in the +/− condition, indicating a higher permeability of the matrix. Incubation of 10-week-old beads in buffer with collagenase MMP-1, lead to a 2.5 fold increase of collagen release in the supernatant in the condition without crosslinks (+/−) than in the two conditions with crosslinks (−/+ and −/−). SOX9 expression was not influenced by addition of BAPN whereas the COL2/COL1 ratio was higher, indicating that inhibition of crosslinks results in more collagen without affecting chondrocyte phenotype significantly.

Conclusions: Modulation of the amount of crosslinking and subsequently collagen deposition, during cartilage tissue regeneration has an effect on the functional properties of the construct. After 5 weeks, more collagen alone led to an increase of stiffness of the matrix. However, when more collagen was deposited after 10 weeks, crosslinks were also needed to improve matrix stiffness. The quality of the matrix indicated by MMP-1 degradation susceptibility is not influenced by the transient crosslink inhibition. These data suggest that modulation of the collagen network may be a feasible approach to induce cartilage regeneration.

A37
CARTILAGE ENGINEERING AND REPAIR: CONTRIBUTION OF HUMAN GLYCOSYLTRANSFERASES IN THE BIOSYNTHESIS OF MATRIX GLYCOSAMINOGLYCANS

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Purpose: Osteoarthritis is the most prevalent chronic human disease characterised by a deterioration of cartilage. Despite its important socio-economic impact, there is, until now, no satisfactory way to treat the disease. Symptomatic treatment with antalgics or anti-inflammatory drugs does not stop the progression of cartilage degeneration, and often presents side-effects, which limit their chronic use. Our goal is to design new therapeutic approaches, keeping in mind that cartilage is one of the rare human tissues which is unable to regenerate. The loss of matrix components, especially glycosaminoglycans (GAG), is an early event of cartilage damage.

Methods: An innovative strategy of cartilage bioengineering consists in overexpression by gene transfer, of glycosyltransferases able to stimulate the anabolic activity of chondrocytes, in order to promote cartilage repair. In that context, the research of our group is mainly devoted to the identification, cloning and structural characterisation of key-glycosyltransferases involved in the biosynthesis of GAG of the cartilage matrix. The work has been especially focused on the human galactose β 1,3-glucuronosyltransferase-I (GalCT-I) which is involved in the final step of the biosynthesis of the common GAG-protein tetrasaccharidic linkage sequence, GlcA β1-3Gal β1-3Gal β1-4Xyl-O-

Results: We have shown that any variation of the GlcCT-I activity in chondrocytes or cartilage explants, either upon overexpression, or, in contrast, by repression with antisense RNA, could increase, or decrease the GAG content in cartilage, respectively. Interestingly, overexpression of this enzyme was able to completely counteract the proinflammatory cytokine, interleukine 1β-induced depletion of GAG. The neosynthesised GAG were qualitatively similar to those present in the original cartilage ma-
ties of 3, 10, and 70 kDa molecular weight fluorescent dextran struct. Diffusion experiments, using image analysis data fitted custom-built bioreactor system and the growing cartilage constructs were 2-3 orders of magnitude lower than that in water.

Results:

Consumption rate estimates were obtained either from the existing literature or were computed from measurements made using a purpose-built oxygen consumption cell. The superiority of ACS to both HA and saline was statistically significant for all outcome measures and all time points. The mean improvement for patients treated with HA and saline was half that in the ACS-group (p < 0.001). No significant differences between HA treatment and saline-injections (p > 0.05, at all time points and all outcome measures) were recorded. Frequency of adverse advents (AE) was comparable in the ACS- and saline-group (p > 0.05), whereas there were significantly more AE in the HA-group (p < 0.05 for the comparison with ACS and saline).

Conclusions: Patients with OA of the knee treated with intra-articular injections of ACS showed significantly better clinical improvement during 26 weeks of observation compared to patients injected with HA or saline. The results demonstrate that ACS is effective and well tolerated for treating chronic, idiopathic OA of the knee. So far, the efficacy of ACS is defined through chondroprotective, or even chondroregenerative, sequelae.

A39
TREATMENT OF KNEE OSTEOARTHRITIS WITH AUTOLOGOUS CONDITIONED SERUM (ACS)
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Purpose: Common strategies in the treatment of OA do not address most of the underlying pathomechanisms. Several biologically based, local therapies are either in clinical use or in development. A new therapy based on the intra-articular injection of autologous conditioned serum (ACS) has shown clinical benefits in animal and human studies. In the present study, the clinical efficacy and safety of intra-articular injections of ACS were compared to intra-articular hyaluronan (HA) and saline in patients with confirmed knee OA.

Methods: ACS is generated by incubating venous blood with medical grade glass beads. Peripheral blood leukocytes produce elevated amounts of endogenous anti-inflammatory cytokines, such as interleukin-1 receptor antagonist (IL-1Ra), that are recovered in the serum.(1)

In a prospective, randomized, patient- and observer-blind, saline-controlled trial with three parallel groups, 399 patients with knee OA were included with the intention to treat (ITT) analysis. Efficacy was assessed by patient-administered outcome instruments (WOMAC, VAS, SF-8, GPA) after 7, 13 and 26 weeks. The frequency and severity of adverse events were used as safety parameters.

Results: In all treatment groups, intra-articular injections produced a significant reduction in WOMAC-scores and weight-bearing pain (VAS), as well as an improvement in the health-related quality of life. However, the positive therapeutic responses to ACS were stronger compared to other treatment modalities. The superiority of ACS to both HA and saline was statistically significant for all outcome measures and all time points. The mean improvement for patients treated with HA and saline was half that in the ACS-group (p < 0.001). No significant differences between HA treatment and saline-injections (p > 0.05, at all time points and all outcome measures) were recorded. Frequency of adverse advents (AE) was comparable in the ACS- and saline-group (p > 0.05), whereas there were significantly more AE in the HA-group (p < 0.05 for the comparison with ACS and saline).

Conclusions: Patients with OA of the knee treated with intra-articular injections of ACS showed significantly better clinical improvement during 26 weeks of observation compared to patients injected with HA or saline. The results demonstrate that ACS is effective and well tolerated for treating chronic, idiopathic OA of the knee. So far, the efficacy of ACS is defined through improvement in clinical signs and symptoms, particularly pain. It remains to be determined whether there are disease-modifying, chondroprotective, or even chondroregenerative, sequelae.