in comparison with OA, which probably translated into a significantly increase of cartilage OARSI scoring. Additionally, DE-genes within TGF-β and Notch signaling were detected, suggesting their involvement in the regenerative outcome. Interestingly, a high number of transcriptional events occurred in subchondral bone due to joint distraction, mostly concerning down-regulation of DE-genes, highlighting the importance of this tissue in the regenerative effect of distraction on OA cartilage.
Conclusions: This study demonstrates for the first time that distraction initiates a transcriptional regulation already in an early phase, mostly concentrated in subchondral bone and cartilage. Importantly and contrary to the current wide extended opinion, this work demonstrates that a high ECM turnover is present in the early stages of regeneration initiated by temporary joint distraction, which could facilitate the production of a new healthy ECM in a later phase.

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INJECTABLE ENZYMATICALLY CROSS LINKABLE HYDROGELS: A MINIMALLY INVASIVE CELL FREE APPROACH TO REGENERATE DAMAGED ARTICULAR CARTILAGE

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Purpose: Focal cartilage defects as a consequence of trauma are a major risk factor for the development of early onset osteoarthritis. These defects still pose a largely unresolved problem for the treating physician. Previously, we have developed an injectable in situ gelating hydrogel that can be applied in an arthroscopic procedure to fill up cartilage defects by simple injection. These hydrogels consist of hyaluronic acid - tyramine and dextran - tyramine conjugates that cross link in in a cell-friendly enzymatic, peroxidase-based reaction, initiated by non-toxic concentrations of H2O2. During the cross linking reaction the hydrogels co-valently attach to the cartilage resulting in strong bonding and fixation of the hydrogel in the defect. These hydrogels possess chemoattractant properties facilitating the ingrowth of cells as demonstrated in an ex vivo chondral plug model opening the possibility for cell-free cartilage repair. The aim of this study is to test the use of these injectable hydrogels for cartilage repair in an orthotopic chondral defect rabbit model side-by-side compared with autologous chondrocyte implantation. In addition, we evaluated the concept in an equine model for focal cartilage defects.
Methods: Three male rabbits were sacrificed to establish cultures of primary human chondrocytes for implantation purposes. In a pilot rabbit experiment skeletally mature female rabbits were operated under anesthesia and two 4mm wide chondral defects were created in each knee joint. The defects were left untreated, filled up with hydrogel only, or with hydrogel prior mixed with chondrocytes. The various combinations of hydrogel precursors were injected in a liquid state in the defect and left to settle in a mild enzymatically mediated cross linking reaction which took place within less than 20 seconds. Rabbits were sacrificed 4 weeks and 10 weeks after treatment and tissues were collected for histology. In a pilot experiment two horses were operated under general anaesthesia in a fully arthroscopic procedure. In each knee joint, 5mm wide chondral defects were created. These defects were in the same arthroscopic procedure completely filled with the hydrogel. Synovial fluid was collected after 1, 2, 3, 5, 7 and 14 days after surgery. After two weeks horses were humanely euthanisized and tissue was processed for histology.
Results: In pilot experiments in rabbits, chondral defects were completely repaired using the injectable hydrogels after 10 weeks of surgery. Cell-free hydrogels appeared as efficient as cell-containing hydrogels. The data are now confirmed in a larger study group in which treatment with hydrogels is compared to microfracture. In the equine model we demonstrated that the injectable hydrogels could be used to fill up focal chondral defects in an completely arthroscopic procedure. Synovial fluid sampling demonstrated a clinically not relevant small increase in white blood cell count and protein count in the first 2 days after surgery which returned to base-line after 3 to 5 days. Clinical examination and follow up of the operated joints demonstrated normal response to arthroscopic surgery: no adverse effects were noted demonstrating the safety of the procedure. The horses were able to make functional use of their treated legs within a few days and walked normally 2-weeks after surgery. At this time point visual inspection demonstrated the presence of hydrogels in each of the defects. Histological examination demonstrated the presence of cell layers on top of the hydrogel and invasion of cells into the hydrogel both from the top and the bottom. The invading cells were organized in columns, like in normal cartilage, and stained positive for typical chondrocyte markers. They actively deposited glycosaminoglycans.
Conclusions: This study demonstrates the feasibility of developing an arthroscopic and completely cell-free treatment of chondral defects. It also demonstrates the presence of populations of migratory cells in the traumatized joint. These cells can actively migrate to and invade an appropriate scaffolding material in vivo and start the deposition of cartilage matrix. In the future, this work may translate into a biomaterial based regenerative treatment of osteoarthritis by harnessing the regenerative potential of these migratory cells.

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INTRAARTICULAR DRUG DELIVERY OF ANTI-HMGB1 IN HYALURONAN GELS FOR CARTILAGE REPAIR

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Purpose: Joint pain due to degeneration of cartilage tissue in osteoarthritis is a major problem affecting a large part of the elderly population and there are no long-term solutions available today. During recent years means suppression of inflammation that stops joint destruction have been developed and there is now a need for new approaches to stimulate repair. This should preferably be done without use of complicated cell therapies, and an opportunity to accomplish such development is provided by the application of new, injectable derivatives of hyaluronan loaded with anabolic cues. The cross linking chemistry applied is a very rapid reaction between carbazate moieties on one component and aldehyde groups in the other, where a solid gel is formed within 30 seconds. HMGB1 is an endogenous molecule released from activated immune cells as well as dying cells with both proinflammatory and osteoclast-activating features. HMGB1 blockade is proven effective in experimental models of diseases such as arthritis, sepsis and drug induced liver injury. The presence of extracellular HMGB1 is reported in OA specimens and there is growing evidence for a role of HMGB1 in the pathogenesis of OA. The aim of this study was to determine the expression pattern of HMGB1 in a mouse model of osteoarthritis. Furthermore we wanted to explore the effect of anti-HMGB1 therapy on cartilage repair in OA using the monoclonal antibody 2G7 and evaluate drug delivery into the joint from HA gels.
Methods: OA was induced in mice by surgically destabilizing the knee joint through anterior cruciate ligament transection. Anti-HMGB1 alone or encapsulated in the gel was injected intraarticularly. After 8 weeks the animals were sacrificed and serum samples taken and the knees processed for histology. Sections were stained with Safranin-O and scored blinded. TUNEL staining for evaluation of apoptosis and immunohistochemistry for markers of inflammation and proliferation were performed.
Results: We demonstrated that cartilage could be rescued by blockade of HMGB1 in the OA model. HMGB1 staining in the cartilage specimens showed intra- and pericellular expression in chondrocytes. The expression of HMGB1 overlapped with proteoglycan depletion of the articular cartilage. HMGB1 was also present in ligaments, synovia and osteophytes already at early time points. A surprising observation was that also the HA gel alone showed improvement in the cartilage score. The gel precursors carry aldehyde and hydrazide functional groups for the gel formation and unreacted components reside inside and on the surface of the final gel with reactive functional groups on the precursors still accessible. The negative impact of endogenous aldehydes, such as 4-hydroxynonenal, on chondrogenesis have been demonstrated in a number of studies and we believe that interaction and neutralization of endogenous aldehydes in the joint cavity may have affected cartilage healing.
Conclusions: The hyaluronan hydrogel has shown promising results for cell free regeneration therapies by local treatment in the joints. We showed that blocking of HMGB1 improved the cartilage regeneration in experimental OA and the hyaluronan gel in itself carried anti-inflammatory properties. The hyaluronan hydrogel system provide biomechanical support with inbuilt anti-inflammatory properties that can be loaded with anabolic factors to promote cartilage regeneration.