Conclusions: Our results show that a transient (24 hour) exposure of cartilage-to-cartilage repair models to Sprifermin improves integration strength and matrix deposition at the interface (as evidenced by contrast-enhanced μCT showing a more uniform attenuation by increase in GAG-containing proteoglycans). In this study, one 24 hour administration weekly for 4 weeks leads to an overall better outcome than one 24 hour treatment over one month. This study represents for the first time a biologic (and in particular an FGF) has improved the integration of cartilage surfaces in a clinically relevant repair model. The findings implicates Sprifermin potential usefulness in surgical procedures such as OATS and in tissue engineering approaches where cartilage like biomaterials will be required to successfully integrate with native cartilage in order to achieve clinical success.

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CHONDROGENESIS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS: EPIGENETIC CAUSES OF LOW HYPERTROPHY INDUCTION

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Purpose: The purpose of this study was to test the in vitro chondrogenic potential and in vivo capacity for cartilage regeneration of human iPS (hiPS) cells.
Methods: Cell culture and differentiation of EB We used the hiPSC line (SBI, cat# SC802A-1) generated by direct delivery of four proteins fused to a cell penetrating peptide. hiPSCs were passaged to Matrigel-coated polystyrene plates and cultured in the defined mTeSR1 medium. To induce mesenchymal differentiation, embryoid bodies were formed and cultured in suspension for 11 days with hESC growth medium in low-attachment culture dishes.
Chondrogenic differentiation: Chondrocytic differentiation was induced by trypsinizing EB to a single cell suspension, diluting cells to a final concentration of $5x10^5$ cells/ml and forming micromass pellets by centrifugation. Micromass pellets were cultured in 15-ml conical tubes for 21 days using DMEM/F12 medium,of supplemented with 1% ITS (insulin-transferrin-selenium), 10-7 M dexamethasone, 50 mM ascorbate-2-phosphate, 50 mM L-proline, 1 mM sodium pyruvate and 10ng/ml TGF-b3.
Analysis for chondrogenic differentiation: After 21 days of in vitro culture, pellets were analyzed for DNA contents, GAG amount, the expression of chondrogenic markers, and the methylation status.
Transplantation:The hiPS-pellets or alginate-hiPSCs constructs were implanted in the osteochondral defect model rat. The rats received daily injections of cyclosporin A to suppress immune responses in rats. After 6 weeks, the rats were sacrificed.
Results: Mesodermal markers increased in EB while undifferentiated ES markers disappeared. After 21 days of chondrogenic culture in micromass pellets, GAG analysis showed that proteoglycan production was significantly greater in chondrogenic pellets than in undifferentiated hiPSCs and EBs. Safranin-O staining demonstrated that the cells in chondrogenic pellets took on the appearance of immature chondrocytes and secreted extracellular matrix. The chondrogenic marker gene and protein expression increased after 21days of pellet culture. The chondrogenic pellets derived from hiPSCs have very low expression of hypertrophic or osteogenic markers. Also, methylation was increased in hiPSCs compared to hBMSCs in the COL10A1 promoter. This result suggested the epigenetic disparity is the cause of the difference in hypertrophy.
When hiPSCs in either pellet state or in alginate hydrogel were implanted in the osteochondral defects created on the patellar groove of immunosuppressed rats, the defects implanted with chondro-induced hiPSCs showed a significantly better quality of cartilage repair than the control defects.
Conclusions: In conclusion, this study provides a proof-of-principle strategy for using hiPSCs as a cell source for cartilage tissue engineering. While successful in vitro induction of chondrogenesis with improved biochemical characteristics were obtained from hiPS cells, the working mechanisms in the implantation of hiPS cells and strategies for further improvement of in vivo cartilage repair with hiPS cells should be investigated in future studies.

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INJECTABLE HYDROGELS FOR CARTILAGE REPAIR

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Purpose: To create an injectable hydrogel that can be used to plaster eroded cartilage surfaces and / or to fill up focal cartilage defects in a minimally invasive arthroscopic procedure. The plaster should protect the damaged cartilage surface against further cartilage erosion and possess an optimized environment for cartilage regeneration.
Methods: Over the last years we have develop an injectable plaster by introducing hydroxyphenyl groups in the backbone of naturally occurring polymers such as dextran, hyaluronic acid and heparin which resemble or normally reside in the extracellular cartilage matrix. This renders an injectable hydrogel which gelates in an enzymatic peroxidase mediated reaction initiated by non-toxic concentrations of H_2O_2.
From *in vitro* testing we know that our plaster is biocompatible and that it supports cartilage formation of incorporated primary chondrocytes, mesenchymal stromal cells or combinations of both. To further improve our injectable plaster we have introduced several cell attracting, cartilage-mimicking and/or chondrogenesis stimulating elements and studied cartilage formation. The adhesive properties of the injectable plaster was compared to clinically used fibrin glue in a customized set up. Finally pilot studies were performed in fresh horse cadavers to test whether the plaster could be applied in focal cartilage defects in an arthroscopic procedure. The repaired joint was manually flexed to study the plaster's resistance to force.
Results: The incorporation of heparin, hyaluronic acid and chondroitin sulphate in a dextran based hydrogel resulted in a chemoattractant plaster facilitating ingrowth of chondrocytes and mesenchymal stromal cells. When chondrocytes were incorporated the addition of these elements potently stimulated neocartilage formation by encapsulated chondrocytes based on histological analysis and gene expression of typical cartilage markers like COL2A, SOX9 and AGC. The binding of the hydrogel to surface of the defect is important for future clinical applications. Therefore we compared our hydrogel to clinically used fibrin gel and found that gel fixation of the injectable plaster is at least 1 order of magnitude stronger than the gluing properties of fibrin gel (Figure 1). In addition, rheology showed that the injectable plaster has improved mechanical properties compared to fibrin gel. Next we tested our injectable plaster in a minimally invasive surgical procedure. Thus the gel was injected via arthroscopic procedure in a full thickness defect in the joint of a horse. Subsequent the knee of the horse was flexed manually to test the injectable plaster which remained in its place (Figure 2). Since this was a success we are currently planning our next *in vivo* study in which the horses will be followed in time to see how the plaster reacts in a full load bearing joint.
Conclusions: We have developed an injectable hydrogel that gelates in situ using a biocompatible enzymatic cross linking reaction and we have shown that this hydrogel is chemoattractant for chondrocyte progenitors and mesenchymal stem cells. We found that gel fixation of the injectable plaster is stronger than the gluing properties of clinically used fibrin gel. Next we demonstrated that this hydrogel can be applied in a minimally invasive arthroscopic procedure in a horse knee and strongly binds to cartilage and subchondral bone by covalent bonding of the hydrogel to the native tissue.
We are now performing experiments in live animals to test the potential of these injectable plasters to facilitate regeneration of damaged cartilage surfaces.

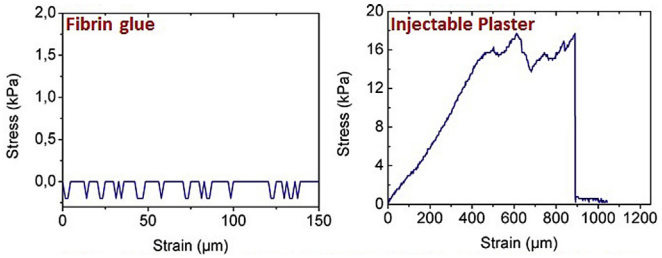

Figure 1. Stress strain diagram of Fibrin gel (left) and the injectable plaster (right). The fibrin gel does not show any up build of forces and is poorly adhering to the defect surface. This in contrast to the injectable plaster.

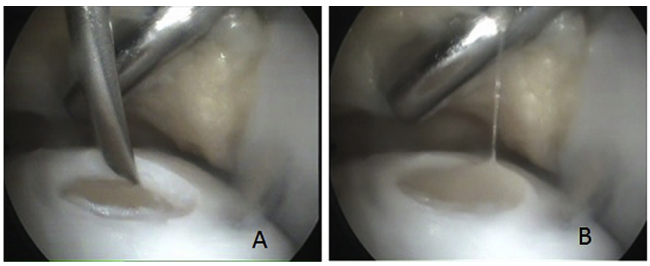

Figure 2. Applying the injectable plasters in a full thickness chondral defect during an arthroscopic procedure. Before (A) and after (B) the injection of the plaster. The initial fluidity ensures complete filling of the defect. The plaster remained fixed at the defect site after manual flexion of the joint.

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USE OF HYALURONAN SCAFFOLD IN COMBINATION WITH FRESH BONE MARROW TRANSPLANTATION OR WITH MICROFRACTURES IN TREATMENT OF CARTILAGE DEFECTS OF THE KNEE JOINT

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Purpose: Microfracture is a well-established procedure in cartilage knee surgery. Currently there are many different techniques utilizing biological implants to support the chondrogenesis. The purpose of this study is to evaluate the medium-term results of the hyaluronic scaffold in combination with fresh bone marrow transplantation or with microfractures in treatment of cartilage defects of the knee joint.
Methods: A new type of hyaluronic acid membrane Hyalofast (Anika Therapeutics, Inc. MA, USA) was used as a CHONDROINDUCTIVE SCAFFOLD that allows attachment and stimulation of mesenchymal stem cells of bone marrow. In study group we used hyaluronan scaffold with fresh bone marrow transplantation and in control group the scaffold was combined with microfractures. Patients were followed using a Socrates Orthopeadic Outcome Software (Ortholink Pty Ltd, Australia) database tool to track changes in function and symptoms by the Lysholm, Tegner and VAS scores along with MRI imaging.
Results: 40 Patients met the inclusion criteria for this study. Both groups consisted of 20 patients. All defects was grade IIIA (n = 10) and IIIB (n = 20) according to the ICRS classification. The mean defect size of the chondral lesions was 2.7 cm (2) (range 1,4–3,1). Defects were localized at the medial (n = 12) and lateral (n = 8) condyle, at the trochlea (n = 12) and at the patella = (8). Based on the imaging evaluation a satisfactory outcome was observed in all cases at estimated intervals. Significant improvement (P = 0,002, wilcoxon rank-sum test) was observed in Lysholm (preop = 61,2 ; 1 year postop = 82,3; 2 year postop = 85,3) Tegner improvement (2,7) and VAS scores. All patients returned to sport activities which they performed before the surgery. No adverse effects related to the implant were observed during the duration of the study. There was no statisctical difference (p = 0,08) in the results between both groups.
Conclusions: The use of hyaluronic acid membrane is considered to be safe and effective procedure for treatment of symptomatic chondral defects of the knee. The data shows that there is no difference between two techniques of utilizing mircofractues or bone marrow transplantation at the defect site. However, further studies with long-term follow-up are needed to determine if the grafted area will maintain structural and functional integrity over time.

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OSTEOARTHRITIS GRADE AND DECISION MAKING IN CARTILAGE REPAIR

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Objective: A recent survey of surgeons that perform microfracture demonstrated that there is a wide variation in technique(1). We also observed that experienced surgeons performing microfracture in cadaveric knees improvised or modified the technique to accommodate sclerotic or osteopenic bone as well as curved surfaces. One concern was that the obtuse approach angle used to approach a surface curving away from the surgeon created a furrow-like elongated hole along the joint surface_ we called this, plowing“. The objective of this expeirment was to determine if plowing affected the integrity of the subchondral bone plate.
Methods: Forty-two 10 x 10 x 15 mm cubes were cut from the lateral femoral condyles of human cadaveric knees. MicroCT (45 micron resolution) was used to characterize the trabecular bone and subchondral bone plate before and after microfracture. Cubes were potted and underwent non-destructive testing (a 0.25% pre-strain at a strain rate of 0.01s-1 (move relative) was applied followed by a dynamic sinusoid of amplitude; 0.05%, frequency; 1Hz, and 20 cycles). Four microfracture holes were created with a 20o awl (Arthrex, Naples FL) at 70, 55 and 40 degrees to the joint surface. After non-destructive testing was repeated all samples underwent compressive testing to failure using preload of 30N and a rate of 0.01s-1 to a peak strain of 25%.
Results: Microfracture significantly reduced the stiffness of subchondral bone ($p < .025$ T-Test) but there was no effect of awl angle on subchondral bone stiffness after microfracture. ($p < .77$, paired T-test). Failure was through barreling and outward displacement of trabeculae than than through through the microfracture holes (2/42 samples).
Conclusions: Microfracture reduced the mechanical properties of the supporting bone in this simplified protocol regardless of awl approach angle. Bone compaction from microfracture is considered deleterious so ‚plowing“ should be discouraged. Creating additional microfracture holes spaced more closely together than currently recommended should be done with caution because bone stiffness would be incrementally affected.