compared to the other subpopulations. CD105 and Runx2 were shown by immunohistochemistry and genetic analysis to have significantly higher expression CD271+ subpopulation than the other subpopulations.
Conclusions: Spheroids formed from CD271-enriched and CD73-enriched MSCs from normal human synovial membranes mimic the native cartilage extracellular matrix more closely than $CD106^+$ MSCs and are possible candidates for use in cartilage tissue engineering. Both cell types have potential for promoting the differentiation of MSCs into chondrocytes, presenting new possibilities for achieving intrinsic cartilage repair.

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DIRECT RAAV-MEDIATED IGF-I OVEREXPRESSION ENHANCES ARTICULAR CARTILAGE REPAIR IN VIVO

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Purpose: Therapeutic gene transfer might be a means to enhance the reparative activities in articular cartilage lesions. We previously reported that recombinant adeno-associated viral (rAAV) vectors are capable of delivering transgene sequences to articular cartilage defects in experimental models over extended periods of time. In the present study, we tested the hypothesis that efficient and sustained overexpression of IGF-I via direct application of rAAV enhances the healing of osteochondral defects created in the knee joints of rabbits *in vivo*.
Methods: rAAV were packaged, purified by dialysis, and titrated by real-time PCR. rAAV-*lacZ* carries the *E. coli* beta-galactosidase (β-gal) marker gene (*lacZ*) controlled by the CMV-IE promoter/enhancer. A human insulin-like growth factor-I (hIGF-I) cDNA was cloned in rAAV-*lacZ* instead of *lacZ* to produce rAAV-hIGF-I. Two osteochondral defects (3.2-mm in diameter) were created in each patellar groove of Chinchilla bastard rabbits (n = 8). Each animal received alternatively 10 μ l rAAV-hIGF-I per defect on one knee (IGF-I-treated defects) and 10 μl rAAV-*lacZ* per defect on the contralateral knee (control defects). At 3 weeks post operation, cartilage repair was assessed based on safranin O/hematoxylin eosin-stained sections using a histological grading system. A total of 145 paraffin-embedded sections (5 μm) were scored independently by two individuals that were blinded with respect to the treatment. Points for each category and total score were compared between the groups using a mixed general linear model with repeated-measures analysis of variance. Indirect immunohistochemical staining was also performed to detect β-gal, hIGF-I, and type-II collagen. The DNA, proteoglycan, and type-II collagen contents of the repair tissue within the defects were measured using Hoechst 33258, by binding to DMMB dye, and by ELISA, respectively. Data are expressed as mean ± SD. The t-test and the Mann-Whitney Rank Sum Test were employed where appropriate.

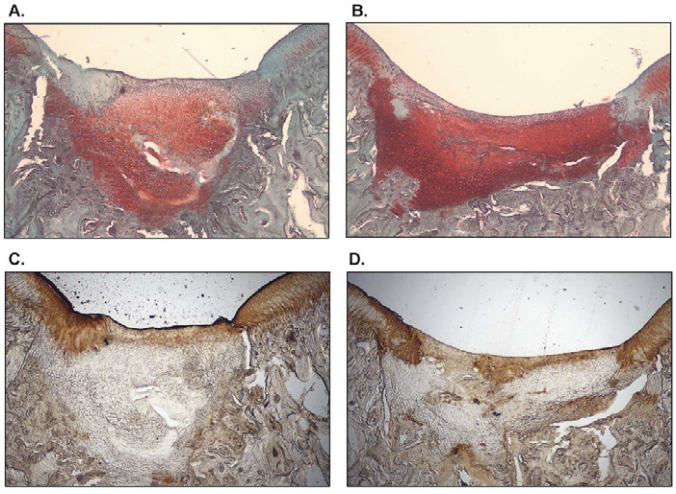

Figure 1. Safranin O staining (A, B) and type-II collagen immunoreactivity (C, D) in osteochondral defects 3 weeks after vector application (A, C: rAAV-lacZ; B, D: rAAV-hIGF-I). Magnification ×2.

Results: Following direct application of the vectors *in vivo*, there were no signs of synovitis, adhesions, or adverse reactions, and no macroscopically descriptive differences between the IGF-I-treated and control knees. β-gal activity was restricted to the control defects, whereas IGF-I expression was present only in the IGF-I-treated defects. After 3 weeks, enhanced tissue healing was observed in the IGF-I-treated defects (Fig. 1). Improved individual parameter scores were observed for defect filling, integration, matrix staining, cellular morphology, defect and surface architecture, new subchondral bone formation (all $P<0.001$) and tidemark ($P<0.01$) of the IGF-I-treated defects, with also a significantly improved total score vis a vis control treatment ($P<0.001$). Immunoreactivity to type-II collagen was more intense and regular in the IGF-I-treated defects (Fig. 1). Biochemical analyses performed on the repair tissue from the defects revealed that treatment with rAAV-hIGF-I promoted a significant increase in the DNA (3.2-fold; $P<0.001$), proteoglycan (1.2-fold; $P = 0.01$), and type-II collagen contents (2.8-fold; $P<0.001$).
Conclusions: The data indicate that IGF-I can be overexpressed in osteochondral defects *in vivo* via rAAV transduction, leading to the production of a recombinant IGF-I factor that is capable of significantly improving the healing of the defects, stimulating both cell proliferation and extracellular matrix synthesis. The results suggest that therapeutic rAAV may have value in enhancing cartilage repair by application to sites of cartilage damage. Further studies are required to evaluate the long-term properties of the repair tissue.

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CELLULAR AND MOLECULAR CHARACTERISTICS OF CHONDROCYTES DERIVED FROM PATIENTS WITH OSTEOARTHRITIS REVEAL THEIR APPLICABILITY IN MATRIX-ASSISTED AUTOLOGOUS CHONDROCYTE TRANSPLANTATION

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Purpose: Cartilage defects arise primarily due to traumatic or degenerative changes and in the medium to long-term often result in osteoarthritis and total joint replacement. One aim of modern orthopaedics is to avoid TJR. For traumatic and small focal degenerative cartilage defects, in the recent years promising treatment options like lavage, abrasion, osteochondral autograft transfer, microfracture, cell-free implants, autologous chondrocyte transplantation and matrix assisted ACT have been developed. However, these therapies cannot completely arrest OA progression and currently are not applied for the treatment of large OA defects. This is partly due to a lack of knowledge regarding the cellular and molecular characteristics of OA chondrocytes. The extracellular matrix is the functional element in cartilage and its degradation is central in the pathogenetic process in OA. In addition, new formation of ECM is crucial in OA chondrocyte based therapies. Therefore, the aim of our study was to provide a more complete picture of the cellular and molecular alterations in OA cartilage and to analyze cartilage formation by OA-chondrocytes.
Methods: Human articular cartilage biopsies were collected from OA patients as well as healthy normal donors. RNA was isolated from the biopsies and subjected to genome-wide microarray analysis. Important results were verified using qPCR and immunohistochemistry. Furthermore, chondrocytes were harvested from such biopsies applying protocols used for ACT. Their chondrogenic potential was studied in high-density pellet and hyaff-11 cultures by proteoglycan and collagen type II staining. Moreover, chondrocytes cultured in monolayer and hyaff-11 scaffolds were subjected to microarray gene expression profiling. Again, expression data were verified by qPCR.
Results: We detected genes already associated with normal cartilage and alterations in OA cartilage but also detected new candidates not previously associated with this disease. Here, the expression of genes coding for collagens (COL8A2, COL13-15A1) or related to bone formation (CLEC3B, CDH11, GPNMB, CLEC3A, CHST11, MSX1-2) was significantly higher in native OA cartilage than in native ND cartilage. In 3D hyaff-11 cultures, chondrocytes from ND and OA donors secreted comparable amounts of ECM components like proteoglycans and collagen type II. Expression of cartilage marker genes (ACAN, COL2A1, COMP, SOX9) and genes involved in matrix synthesis (BGN, COL9A2, COL11A1) was highly induced in 3D ND and OA