Purpose: Site-1 protease (S1P) is a proprotein convertase that is primarily known for processing of latent, endoplasmic reticulum (ER) membrane-bound transcription factors SREBPs and ATF6 to their free and active form. SREBPs are involved in cholesterol and fatty acid homeostasis; ATF6 is involved in ER stress response. Cartilage-specific knockout of S1P in mice ($S1P^{cko}$) results in poor cartilage with a drastic decrease of collagen type IIB (Col IIB) in the matrix and a complete lack of endochondral bone formation. Ultrastructural analysis of the cartilage shows engorged and fragmented ER characteristic of ER stress. $S1P^{cko}$ chondrocytes would be expected to lack both active SREBPs and ATF6 proteins. The goal of this study is to identify the requirement of SREBPs- and ATF6-related pathways for normal Col IIB deposition in the cartilage
Methods: RNA was extracted from the cartilage of E16.5 $S1P^{cko}$ and wild type (WT) littermates followed by genome-wide expression profiling using Illumina MouseWG-6 v2 Expression BeadChip, and differential gene expression was analyzed by 2-way ANOVA. The expression profiling was further confirmed by qPCR. Col IIB processing in $S1P^{cko}$ mice was analyzed by immunofluorescence. Knockout mouse models of genes down-regulated in $S1P^{cko}$ chondrocytes were analyzed for Col IIB deposition. Cartilage-specific deletion of S1P was also analyzed temporally in a postnatal S1P ablation model via in situ hybridization and immunofluorescence to follow the relationship between S1P ablation and Col IIB entrapment in chondrocytes.
Results: Genome-wide expression profiling followed by qPCR analyses demonstrated that ATF6-directed ER stress response was normal in $S1P^{cko}$ mice. However, fatty acid and cholesterol synthesis was down-regulated in $S1P^{cko}$ with the largest reduction seen in expression of stearoyl-CoA desaturase 1 (Scd1) gene. However, $Scd1^{-/-}$ knockout mice did not display any defects in Col IIB deposition. Double labeled immunofluorescence analysis demonstrated the retention of Col IIB in the ER of $S1P^{cko}$ chondrocytes, but not that of COMP and aggrecan. Temporal studies with postnatal S1P ablation demonstrated that Col IIB retention inside the chondrocyte is almost immediate on S1P ablation, which was followed later by chondrocyte apoptosis. In double-labeled immunofluorescence studies targeting Col IIB triple helical domain and the Col II N-propeptide, the WT exhibited signals from the triple helical domain primarily in the matrix and those from the N-propeptide inside the cell. In $S1P^{cko}$ however, signals from the triple helical domain and the N-propeptide overlapped inside the cell, suggesting abnormal processing of the Col IIB protein. Western blotting for the Col II N-proteinase ADAMTS3 demonstrated the retention of ADAMTS3 in $S1P^{cko}$ chondrocytes that was not seen in WT cells
Conclusions: Our studies demonstrated that S1P plays a pivotal role, direct or indirect, in Col IIB maturation. The retention of Col II N-terminus and Col IIB triple helical domain along with ADAMTS3 in $S1P^{cko}$ chondrocytes indicates a role for S1P in Col IIB processing, in the absence of which chondrocytes are unable to secrete Col IIB into the matrix. Even though cholesterol and lipid homeostasis pathways are down-regulated in $S1P^{cko}$, the lack of any bone or cartilage phenotype in $Scd1^{-/-}$ knockout mice suggests that S1P does not play this role via SREBP processing. As ATF6-related pathways are not down-regulated in $S1P^{cko}$ and ER stress response was secondary to Col IIB retention, ATF6-related pathways are not the primary cause for Col IIB anomalies. These data along with the retention of ADAMTS3 in $S1P^{cko}$ chondrocytes suggest additional proprotein convertase activities for S1P, besides transcription factor processing, that are essential for Col IIB processing and cartilage development.

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INORGANIC POLYPHOSPHATE EXHIBITS ANABOLIC EFFECTS ON ARTICULAR CARTILAGE

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Purpose: Osteoarthritis (OA) is a group of diseases characterized by the loss of articular cartilage as a result of an imbalance between anabolic and catabolic processes, as well as tissue calcification. Current OA therapies aim to reduce symptoms but do not modify the progression of the disease. Inorganic polyphosphate (PP) is a linear polymer of orthophosphate residues linked by energy-rich phosphoanhydride bonds. It is found ubiquitously in biological systems from bacteria to animal cells and is present in relatively large quantities in articular cartilage and bone. PP is known to inhibit tissue calcification. The purpose of this study was to determine if PP treatment would have an anabolic effect on cartilage. This was evaluated both *in vitro* and *in vivo*.
Methods: Articular cartilage was obtained from bovine metacarpal-phalangeal joints and either maintained in explant culture or used for chondrocyte isolation. Cells harvested by enzymatic digestion were seeded on membrane inserts ($1x10^6$ cells/insert). The 3D cultures were maintained under standard tissue culture conditions in the presence or absence of PP for various times up to 4 weeks. Resulting tissues were analyzed for DNA, glycosaminoglycan (GAG) and collagen synthesis and accumulation, gene expression and exopolyphosphatase activity. Full thickness cartilage explants were treated similarly but harvested after 1 week. To evaluate the effect of PP on the progression of OA, left-medial menisectomy was performed on the knee of 20 male Hartley guinea pigs (3 months of age). Starting the day following surgery, the animals received biweekly intra-articular injections (100µl) of either: 1) 4mM PP in PBS, or 2) PBS alone (10 animals/ condition). After 2 months, the animals were sacrificed and the joints, following decalcification and paraffin embedding, were evaluated histologically using the OARSI guinea pig grading scale.
Results: PP stimulated GAG and collagen accumulation by chondrocytes in a concentration and chain length dependent manner. The optimal PP chain length for matrix accumulation was 45 phosphate units. This effect of PP on matrix accumulation was reversible as exopolyphosphatases were produced by chondrocytes that can cleave the end phosphate of PP chains. Similar concentrations (on a phosphate basis) of orthophosphate and inorganic pyrophosphate had no effect on matrix accumulation by chondrocytes. Collagen type II, aggrecan and sox9 gene expression were not altered by the PP treatment but proteoglycan and collagen synthesis were both significantly increased by the treatment. A time course study demonstrated increased DNA content in cultures treated with PP with time, albeit at a decreased rate compared to untreated cultures. Cartilage explants responded similarly to PP treatment as the chondrocyte cultures. Intra-articular injections of PP were chondroprotective as the histological OA score was significantly decreased in the treated OA guinea pig model compared to that of animals treated with the PBS carrier (PP treated: 7.4 $\pm$ 3.09; Carrier: 16.9 $\pm$ 3.55; $p<0.05$; Mann-Whitney test; $n=10$).
Conclusions: PP was shown to be chondroprotective in a menisectomized guinea pig model of OA. *In vitro* studies suggest this effect is mediated at least in part by stimulating matrix production by chondrocytes. Further study is ongoing to optimize PP delivery *in vivo*.

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SECOND GENERATION CHARACTERIZED CHONDROCYTE IMPLANTATION FOR THE TREATMENT OF CARTILAGE LESIONS IN THE KNEE

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Purpose: To evaluate short-term clinical and MRI outcome of the second generation characterized chondrocyte implantation (CCI) for the treatment of cartilage defects in the knee.
Methods: Thirty-two patients aged 15-51 years with sin- gle International Cartilage Repair Society (ICRS) grade III/ IV symptomatic cartilage defects of different locations in the knee were treated with CCI using a synthetic collagen I/III membrane to cover the defect. Clinical outcome was measured over 36 months by the Knee injury and Osteoarthritis Outcome Score (KOOS) and Visual Analogue Scale (VAS) for pain. Serial magnetic resonance imaging (MRI) scans of 22 patients were scored using the original and modified Magnetic resonance Observation of Cartilage Repair Tissue (MOCART) system.
Results: The patients included in this study showed a significant gradual clinical improvement after CCI. The MRI findings of this pilot study were considered to be promising. No signs of deterioration were observed. A complete or hypertrophic filling was observed in 76.5% of the cases at 24 months of follow-up. No preventive effect of an avital membrane on the occurrence of hypertrophic repair tissue was observed on MRI. Three failures were observed among the 32 patients until now (9.4%).
Conclusions: This investigation provided useful information on the efficacy of this treatment. The short-term clinical and MRI outcome are promising. Large-scale and long-term trials are mandatory to confirm the results and the reliability of this procedure.