peak and activation of the executioner caspase-3 was evaluated. Results show that preincubation for two hours with all inhibitors significantly decreased the hypodiploid DNA peak by treatment with TNF-α+Ro at 24 hours (4,03±3,13%, 7±4,23%, 3,6±1,09%, and 3,14±0,77% with caspase-3, caspase-3/7, caspase-8 and caspase general inhibitor, respectively, n=4, $p<0,0001$). When caspase-3 activation was evaluated by western blot, it was found that preincubation of caspase-8 inhibitor was effective to prevent caspase-3 induced by TNF-α+Ro. However, caspase-8 inhibitor not prevent bcl-2 decreased induced by TNF-α+Ro or IL-1β+Ro.
Conclusions: These results confirm that the cytokines TNF-α and IL-1β differently regulate machinery apoptotic activation in human chondrocytes independently of the apoptosis model employed and this difference is dependent on caspase-8 levels. These data could be important for a better understanding of the participation of TNF-α and IL-1β in the pathogenesis of cartilage degradation.

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KARL-FISCHER-TITRATION AND COULOMETRY FOR MEASUREMENT OF WATER CONTENT IN SMALL CARTILAGE SPECIMEN

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Purpose: This study was aimed to evaluate the sufficiency of the Karl-Fischer Titration and coulometry for measurement of water content in small intact and defect cartilage specimen.
Methods: Cartilage from the main weight bearing zone of the medial condyle of thirty-eight fresh sheep knees were used. Twenty condyles had an intact cartilage. Defects (14 grade I and 4 grade II defects) were found in the rest. The mechanical hardness was determined as shore A. Than cartilage specimens of about 5mg were analyzed in special devices for moisture measurement. During measurement the specimen primary were holds at room temperature to evaluate the water liberation by spontaneous humidification. After this, the specimens had undergone continuously heating up to 105°C. The actually measurement was performed in an electric measurement cell (coulometry). An electrode was laminated with hygroscopic phosphorus pentoxide. In electrochemical reaction H and O is liberated from the electrode. The requirement of electric energy correlates with the amount of water in the specimen.
Results: The water content in intact cartilage was 66.9%. Grade I (72.6%) and grade II defects (77.8%) defects had a significant higher water content. In cartilage defects was observed a significant higher and faster spontaneous humidification at room temperature. Total water content as well as spontaneous water humidification correlated with a significantly decrease of mechanical hardness.

Table 1. Total and superficial water content in intact and degenerated cartilage in correlation to stiffness

Defect grade	0	I	II	p<
n	20	14	4	0.000
Superficial water content [%]	3.6	4.9	5.9	0.001
Total water content [%]	66.9	72.6	77.8	0.000
Stiffness [shore A units]	90.5	64.1	65.2	0.000

Conclusions: The experimental design (a combinated method of thermogravimetry, Karl-Fischer-titration, and coulometry) was sufficient for evaluating the water content in small cartilage specimen. It is also possible to measure the temperature-dependent water liberation from the cartilage specimens.

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THE EFFECTS OF SYNTHETIC TRITERPENOIDS ON SZP SYNTHESIS IN ARTICULAR CHONDROCYTES

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Purpose: The articular chondrocytes of superficial zone secrete superficial zone protein (SZP) which is homologous to lubricin, and functions as a lubricant in articular joints. Camptodactyly-arthropathy-coxa vara-pericarditis syndrome is known to be caused by mutations in the PRG4 gene which encodes SZP. Understanding of the regulators of SZP synthesis is important for

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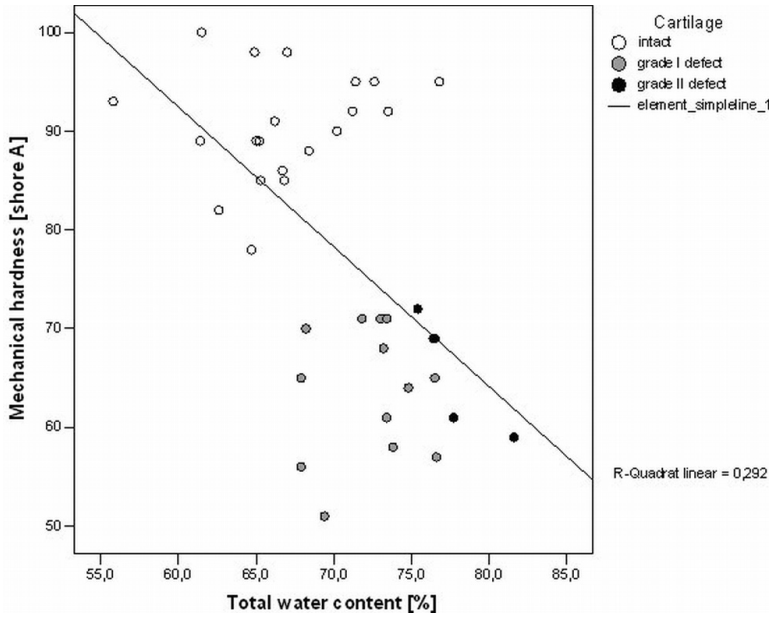

investigating disease, homeostasis of articular joint, and tissue engineering of functional superficial zone of articular cartilage.
Synthetic triterpenoids (TPs) are potent multifunctional molecules. However, their effects in various types of cells are still unclear. TP-151 and its derivative, TP-235 are synthetic triterpenoids and reported to possess capacities to modulate transforming growth factor-β (TGF-β)/Smad signaling in several types of cells.
We hypothesized that synthetic triterpenoids could regulate SZP synthesis in articular chondrocytes because TGF-β is known to possess a capacity to up-regulate SZP synthesis. The aim of this study is to investigate whether synthetic triterpenoids regulate SZP synthesis in articular chondrocytes.
Methods: Bovine calf stifle joints were used in this study. The superficial zone of articular cartilage was harvested using a dermatome. The cartilage slices were digested with 0.2% collagenase P in culture medium. Isolated chondrocytes were plated in monolayer and cultured in the serum-free chemically defined media. Various concentrations of synthetic triterpenoids were added into the cell culture. We used four TPs, TP-151, TP-155, TP-235, and TP-319 in this study.
As most of SZP is secreted into the culture medium, supernatants were harvested as samples and the SZP synthesis was analyzed by SDS-PAGE and Western blotting using a monoclonal antibody S6.79. SZP accumulation into the culture medium was also quantified by ELISA using purified SZP as standard.
Results: TGF-β1: Recombinant human TGF-β1 up-regulated SZP synthesis in a dose-dependent manner (0.4-120 pM).
TPs at higher concentrations: All four TPs down-regulated SZP synthesis in a dose-dependent manner at higher concentrations (100-1000 nM). Concurrent treatment with TGF-β1 (4 pM) also demonstrated the same trend.
TPs at lower concentrations: TP-151 and TP-155 up-regulated SZP synthesis at lower concentrations (0.01-1 nM). TP-235 and TP-319 exhibited no obvious effects in this range.
Conclusions: TP-151 and TP-235 were reported to increase the expression of TGF-β-dependent genes and synergize with TGF-β in this regard at nanomolar concentrations in cells of epithelial and hematopoietic origin. Moreover, they are reported to prolong the activation of Smad 2 induced by TGF-β and enhance the ability of Smad 3. Other investigators reported that TP-151 induced TGF-β1 production and Smad 2 expression in intraepithelial lymphocytes.
However, on the other hand, TP-151 (300 nM) was reported to antagonize Smad-dependent transcription of $CAGA_{12}$-luciferase in chondrocytic cells. Therefore the effects of TPs on TGF-β/Smad signaling is still controversial. Synthetic triterpenoids may have tissue/cell-specific effects on the Smad pathway.
Our results demonstrated biphasic effects of TP-151 and TP-155. The down-regulation of TP-151 at higher concentrations is compatible with the report that 300 nM of TP-151 antagonized Smad-dependent transcription of $CAGA_{12}$-luciferase in chondrocytic cells. Our results suggested that sub-nanomolar concentrations were optimal for articular chondrocytes. The effects of TPs on the other zones of articular cartilage (middle zone and deep zone) are also important targets to investigate.

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5-AMINOLAEVULINIC ACID BASED PHOTODYNAMIC THERAPY CAUSES AN INACTIVATION OF CHONDROCYTES BUT NOT OF BONE CELLS IN OSTEOCHONDRAL GRAFTS

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Purpose: The clinical outcome in osteochondral allografting is limited due to the immunological incompatibility of the osseous portion. A means to improve the clinical outcome consists in the pretreatment of allografts to achieve a devitalized osseous portion while maintaining the function of cartilage. Photodynamic therapy (PDT) is based on the preferential accumulation of a photosensitizing drug in a target tissue and its activation by light. Formation of cytotoxic products leads to selective destruction of cells containing the photosensitizer. Cell culture experiments with cartilage and bone cells revealed that chondrocytes remained viable and functional after 5-aminolaevulinic acid (5-ALA) based PDT, whereas primary osteoblast-like cells showed a strong decrease in function and survival. This lead to the hypothesis, that 5-ALA-PDT can be applied to devitalize the osseous portion of an osteochondral graft selectively, while maintaining cartilage function. The aim of this study was to expand the cell culture experiments to an *ex vivo* tissue culture model.
Methods: Cylindrical osteochondral grafts (5x10 mm) were harvested from porcine humeral heads. After incubation for 4h at 37°C in 1mM ALA the grafts were illuminated with 5, 10 and 20J/cm^2. Control grafts were fixed immediately after harvest ("direct fixation"), incubated in 1mM ALA ("ala-dark") or illuminated with 20 J/cm^2 in the absence of incubation with 5-ALA ("light alone"). After treatment, the grafts were cultured at 37°C for 4h. Subsequently the grafts were either fixed in paraformaldehyde or stored for further 16h at 4°C before fixation. The function of the cells was assessed by *in situ* hybridization for mRNAs encoding collagen type I (colα_1(I); osteoblasts) and type II (colα_1(II);chondrocytes).
Results: The surface of trabeculae bone covered with osteoblasts positive for mRNA encoding colα_1(I) was 0.52±0.34 ("ala-dark"), 0.57±0.13 (5J/cm^2), 0.47±0.28 (10J/cm^2), 0.50±0.32 (20J/cm^2) 4h post PDT and 0.29±0.12, 0.30±0.21, 0.25±0.16 and 0.26±0.13 respectively 20h post PDT (mean±SD, % of total trabecular surface, relative to "direct fixation"). The area covered with chondrocytes positive for mRNA encoding colα_1(II) was 0.93±0.08, 0.96±0.03, 0.67±0.09, 0.70±0.15 respectively 4h post PDT and 0.94±0.08, 0.76±0.12, 0.61±0.17 and 0.38±0.17 respectively 20h post PDT (mean±SD, % of total cartilage, relative to "direct fixation"). The loss of colα_1(I) expression in osteoblasts 20h post PDT in the groups "ala-dark", 5,10,20 J/cm^2 compared to the control "direct fixation" was statistically significant. The loss of colα_1(II) expression in chondrocytes was statistically significant for the groups 10 and 20J/cm^2 compared to the control "direct fixation" 4h and 20h post PDT.
Conclusions: Bone cells become non functional during the 24h experimental protocol. There is no additional effect of 5-ALA-PDT on bone cells, may be due to the spontaneous cell death occurring in these cells in the culture or either 5-ALA could not sufficiently diffuse through the osseous tissue, the light penetration was not sufficient, or a combination of both. 5-ALA-PDT caused an unexpected loss of functional chondrocytes within the cartilage maybe due to an increased sensitivity caused by the characteristics of this experimental setup. Chondrocytes remained functional in the control group for tissue culture whereas osteoblasts were sensitive to the applied tissue culture conditions. The aim of the study to obtain a devitalized osseous portion but functional cartilage could rather be achieved by the applied tissue culture conditions than by 5-ALA-PDT.