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Integrin α2β1 influences bone remodeling by regulating osteoblastogenesis via BMP signaling

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Introduction Integrin α2β1 is classified to the integrin family, a group of heterodimeric transmembrane receptors with important roles in cell adhesion, cell-cell communication, and interplay of cells with the extracellular matrix. Furthermore, it is one of four collagen-binding integrins and the major receptor for collagen type I in bone tissue. Our group showed that the loss of integrin α2β1 facilitate the expression of collagen type I and protects against age-related osteoporosis [1]. In this study, we investigated the impact of integrin α2β1 in bone metabolism and fracture repair with perspective for clinical use.

Material and Methods Using constitutive knockout mouse model [2] we examine the effect of integrin α2β1 on collagen synthesis and mineralization in bone tissue. We applied biochemical assays, like gene expression level, ELISA and immunofluorescence staining, histological techniques, like Alcian blue staining, and μCT scans.

Results Integrin α2β1-deficient mice exhibit an accelerated fracture repair involving enhanced collagen synthesis, faster callus formation and mineralization. Additionally, the BMP signaling, a key factor for osteogenesis and mineralization, shows an earlier onset and enhanced activation during osteoblastic differentiation in vitro. The mineralization develops faster, the BMP-2 expression level and extracellular located protein level is earlier enhanced, and the downstream signaling via activation of SMAD1 is earlier elevated.

Conclusion These results point out that the absence of integrin α2β1 leads to an improved fracture repair by accelerated signaling of pro-osteogenic factor BMP-2. Therefore, we conclude a regulating role of Integrin α2β1 on bone metabolism. This recent insight may facilitate new therapeutic approach for fracture repair and non-unions.

Conflict of Interest All named authors declare that they have no conflict of interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

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Deletion of the mechanosensitive protein Piezo1 in *Col2a1*-expressing cells impairs postnatal trabecular bone formation

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Introduction Recently, we and others identified Piezo1 as the long-sought mechanosensor in osteocytes [1–4]. Mice with an osteocyte-specific deletion (*Dmp1-cre*) of *Piezo1* are osteoporotic and their long bones do not respond to mechanical stimulation [1]. Furthermore, osteoblast-specific deletion (*Runx2-cre*) led to an even more severe skeletal phenotype. Adult mice had no secondary spongiosa and rib fractures occurred in two-week-old mice. Most interestingly however, the deletion of *Piezo1* in chondrocytes (*Col2a1-cre*) led to a similar phenotype as in mice with an osteoblast-specific deletion. We therefore examined the development of the skeletal phenotype in $Piezo1^{Col2a1cre}$ mice starting from birth up to 12 weeks of age.

Materials and Methods The skeletal phenotype of $Piezo1^{Col2a1cre}$ and $Piezo1^{fl}$ littermates was analyzed using contact radiography, histomorphometry, μCT and growth plate measurements at different ages.

Results While $Piezo1^{Col2a1cre}$ mice did not differ from their $Piezo1^{fl}$ littermates immediately after birth, they showed a strongly decreased bone volume in the lumbar spine starting from two weeks of age, while growth plate thickness was only minimally affected at three weeks of age. Additionally, we observed numerous rib fractures starting from one week of age.

Conclusion *Col2a1cre* mice are typically used as a model to target chondrocytes. However, deletion of the mechanosensitive protein *Piezo1* in these mice only minimally affected growth plate cartilage. Surprisingly, however, $Piezo1^{Col2a1cre}$ mice developed severe osteoporosis. Therefore, we hypothesize that Piezo1 plays a role in chondrocyte-to-osteoblast transdifferentiation, which will be analyzed more in-depth in the future.

Conflict of Interest None.

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Single cell RNA sequencing identifies mitochondrial respiration as a key factor contributing to extracellular matrix integrity

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Introduction During endochondral ossification, chondrocytes are in need of constant energy supply to maintain proliferation and matrix deposition. Mitochondrial respiration is one of the major cellular energy source and is described to drive postnatal growth, but the importance for extracellular matrix (ECM) homeostasis is poorly understood.

Material and Methods Cartilage morphology of 28 days old mice with a cartilage specific genetic inhibition of the respiratory chain (Col2Cre-Twinkle) was studied by immunological and histological methods. Individual transcriptomes of chondrocytes were analysed by single cell RNA sequencing (scRNA-seq) and proteome analysis was applied to define consequences for ECM composition. Properties of the ECM were characterised by ultrastructural analysis and atomic force microscopy.

Results Inactivation of the respiratory chain in chondrocytes results in disorganization and expansion of femoral head cartilage of Col2Cre-Twinkle mice. scRNA-seq analysis demonstrates that mitochondrial DNA encoded genes are specifically decreased in the nonarticular chondrocytes population accompanied by a changed expression of ECM-related genes. Among those thrombospondin-1 and matrilin-1 are accumulated in the enlarged disorganized femur head cartilage of mutant mice. The changes in the ECM are associated with an increase of ECM crosslinks and higher stiffness of mature cartilage.

Conclusion The results show that mitochondrial respiration is an important biochemical cue for ECM organisation and mechanostability of mature cartilage.

Loss of adipogenic dickkopf-1 increases trabecular and cortical bone mass by promoting bone formation in male mice

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Introduction Osteoporosis is a frequent disease characterized by a decrease of bone mass and quality leading to a higher risk of fractures. Dickkopf-1 (DKK1) is an inhibitor of Wnt signaling and thereby inhibits osteoblast differentiation and decreases bone mass. In our previous studies, we showed that osteogenic cells are the main producers of circulating DKK1. Given that osteoblasts and adipocytes share the same precursor mesenchymal stem cell and that bone marrow adiposity increases with age, we investigated the impact of adipogenic DKK1 deletion on bone homeostasis in mice.

Material and Methods The adipogenic deletion of DKK1 was induced in 6-week-old mice through cre recombinase-loxP system (DKK1f/f; AdipoQ-Cre, cKO) by five subsequent injections of tamoxifen (10 mg/kg). Six weeks later, the body weight and fat pads of cKO and their littermate controls were measured. Bone parameters were assessed by micro-computed tomography, bone turnover markers by using enzyme-linked immunosorbent assays, and biomechanical testing. Each group included 7–20 mice.

Results Female and male adipogenic DKK1 cKO showed a similar body weight compared to control mice. However, males revealed a decrease of the gonadal and subcutaneous fat pads with 16 % ($p < 0.05$) and 46 % ($p < 0.05$), respectively, compared to control mice. While female cKO mice showed no alterations in bone parameters, male cKO mice showed a 61 % increase in femoral trabecular bone volume. The trabecular number increased by 9 %, the trabecular thickness by 11 %, while trabecular separation was decreased by 10 %. The cortical thickness of cKO was increased by 4 %. In the fourth lumbar vertebra, trabecular bone volume was increased by 21 % and bone mineral density by 14 %. Biomechanical tests revealed that cKO mice had a 7 % and 8 % improvement in femoral and vertebra stiffness, respectively, compared to the control group. Male DKK1 cKO mice showed increased serum levels of P1NP (+ 75 %). Serum CTX was elevated by 32 %, while serum TRAcP5b levels were decreased by 16 % in DKK1 cKO mice compared to control mice.

Conclusion Based on this study, DKK1 derived from adipogenic cells appears to influence adipose tissue expansion and signal in a paracrine way to inhibit bone formation and thereby reduce bone mass.

Conflict of Interest No conflict of interest.

Comparison of cell populations in tissue samples of the acetabulum and proximal femur of patients with hip osteoarthritis

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Introduction During hip replacement surgery, bone marrow (BM) containing tissues accumulate as residual products representing potential sources of progenitor cells for regenerative therapies. Therefore, we characterized BM mononuclear cells (BMNC) from the acetabulum and proximal femur of patients with hip osteoarthritis.

Material and Methods BMNC were isolated from tissue samples of 18 predominantly elderly (mean age 64 years), overweight (BMI-average 30 kg/m^2) patients (56 % female). BM mesenchymal stromal cells (BMSC), immune and hematopoietic cells were analyzed by flow cytometry. Besides the ability of BMSC for osteogenic and adipogenic differentiation, colony forming capacities of BMSC and hematopoietic progenitors were determined. Cell-population characteristics were compared between donor-matched acetabular and femoral samples.

Results BMNC density in acetabular samples was nine times lower compared to femoral samples. While there was little difference in the frequency of native BMSC-markers in flow cytometry, tissue progenitor cells from acetabulum exhibited significantly shorter generation doubling times, better colony formation and stronger mineralization in osteogenic differentiation than cells from femur. Despite a much lower $CD45^+$ population and plasma cell fraction ($CD19^+CD38^+CD138^+$), significantly higher proportions of T cells ($CD45^+CD3^+$), especially T-helper cells ($CD45^+CD3^+CD4^+(CD25^+)$), were found in acetabular samples. In contrast, hematopoietic ($CD34^+$) and clonogenic erythroid progenitor cells were increased in femoral samples associated with higher lipid droplet accumulation in adipogenic differentiation assays of BMSC.

Conclusion Acetabular samples revealed a pro-inflammatory cell-population with activated T-helper cells and increased capacity for osteogenic mineralization, whereas femoral samples contained increased cells of the hematopoietic developmental lineage. Whether these differences are due to the influence of osteoarthritis or the different anatomical-functional origin should be clarified in further investigations.

Conflict of Interest Authors declare no competing interests.

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Bone marrow mesenchymal stromal cells can support proliferative fraction of multiple myeloma cells with exhausted stem cell potential

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Introduction Multiple myeloma (MM) is an incurable malignancy of plasma cells accumulating in the bone marrow (BM). MM leads to Myeloma Bone Disease characterized by bone destruction missing new bone formation. BM appears to govern MM cell survival. We investigate the role of BM mesenchymal stromal cells (MSCs) and therapeutics on MM cell behavior.

Material and Methods MSCs were isolated from BM of patients undergoing total hip arthroplasty. MM cells (OPM-2, U266, AMO-1, MM1.S) were analyzed after 72 h of co-culture or treatments with bortezomib (BTZ). Proliferation and cell cycle progression were assessed by flow cytometry. Senescence-related enzyme activity was examined by histological staining. Clonogenic activities were explored by methylcellulose-based assay.

Results Presence of MSCs increased the proliferation rate of MMs, decreased frequencies of CD38 + CD138 + MM cells in SubG1 phase while elevating the frequency of the Ki67high fraction suggesting a support of the proliferative non-adherent fraction in the co-culture system. Cell cycle regulators, p16 and p53 showed distinct trends in mRNA expression between different MM cell lines. Co-culture led to an upregulation of ATP-binding cassette transporter (ABCB1) and a decrease in glutathione S-transferase pi (Gstp1) mRNA expression in MMs. BTZ treatment increased clonogenicity of MM cells. Increase of β-galactosidase activity in MSCs after co-culture suggests MM-MSC interactions may contribute to the senescent profile of MSC.

Conclusion Our results imply that MSCs bring additional complexity to MM populations with distinct stem cell-related and chemoresistance properties. Further investigation will elucidate the mechanistic background of MSC-mediated effects.

Conflict of Interest The authors confirm there was no conflict of interest.

Femoral cortical bone from type 1 diabetes mellitus individuals exhibit impaired osteocyte viability in the periosteal region

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Introduction Type 1 diabetes mellitus (T1DM) patients have an up to 7-fold higher hip fracture risk compared to healthy individuals. The underlying mechanism of impaired bone quality in T1DM is unknown. Osteocytes play a pivotal role in mechanosensation and maintain skeletal homeostasis. Micropetrosis is osteocyte-lacuna mineralisation indicating previous osteocyte death and can delimit the lacuno-canalicular network, possibly resulting in changes in bone quality contributing to increased bone fragility in T1DM.

Material and Methods We analysed the anterior quadrant from femoral cortices of the mid-diaphysis from 25 individuals (T1DM n = 9, 52.5 ± 12.0 years; control n = 16, 51.2 ± 10.2 years) by quantitative backscattered electron imaging (qBEI). We determined bone mineral density distribution (BMDD) and mineralised osteocyte-lacunae at the periosteal and endocortical region.

Results Analysis of BMDD showed a similar degree of mineralisation in T1DM compared to the controls. However, we found two types of mineralised lacunae: fully mineralised and partly mineralised lacunae. We found a higher number of fully mineralised lacunae in the periosteal region in T1DM compared to controls ($p = 0.038$). Moreover, we distinguished the preferred site of micropetrosis accumulation by analysing interstitial and osteonal bone. This anatomical subdivision showed a higher amount of mineralised lacunae within osteons of the periosteal region in T1DM in comparison to controls ($p = 0.049$).

Conclusion Our data revealed that T1DM is associated with an accumulation of mineralised osteocyte-lacunae. Specifically, within osteons at the periosteal region, osteocyte viability and micropetrosis are more apparent. This finding points to reduced viability of osteocytes in T1DM, which may, in turn, affect bone remodelling and microdamage accumulation.

Conflict of Interest I declare no conflict of interest.

Inhibition of cyclin-dependent kinase 5 improves fracture healing in mice

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Introduction Systemic drug therapies to enhance bone formation during fracture healing are limited. Recently, we determined that pharmacological inhibition of cyclin-dependent kinase 5 (Cdk5) with roscovitine increases bone mass by enhancing osteoblastogenesis in mice (unpublished data). In this study, we investigated whether the inhibition of Cdk5 with roscovitine also improves fracture healing in mice.

Material and Methods The animal experiment was approved by the Regierungspräsidium Tübingen, Germany (Nr. 1402). 12-week-old male BALB/cAnNCrl mice received a unilateral femur osteotomy stabilized by an external fixator. Starting on the day of surgery, mice received injections of either vehicle (PBS/DMS/Solutol 17:1:2) or roscovitine solution (150 mg/kg, Selleckchem) intraperitoneally every second day until the day of sacrifice (14- and 23-days, n = 5–8 per group). Bone healing was evaluated using biomechanical testing (three-point bending), micro-computed tomography (μCT) and histomorphometry analyses. Statistics: Unpaired two-tailed student's *t*-test (GraphPad Prism 9).

Results On day 14 post fracture, histomorphometric analysis revealed a significant increase of bone content in the fracture callus from roscovitine-treated mice compared with the vehicle-treated controls (+ 87 %, $p < 0.05$). On day 23, μCT analysis showed a significant increase in bone volume fraction (BV/TV, + 22 %, $p < 0.05$), bone mineral density (BMD, + 16 %, $p < 0.05$) and bending stiffness (+ 32 %, $p = 0.08$), which was further confirmed by the histomorphometric analysis.

Conclusion Our results demonstrate that systemic administration of the Cdk5 inhibitor, roscovitine, improves fracture healing in mice, and suggests *Cdk5* as a promising target for the development of systemic osteoanabolic therapies, considering that Cdk inhibitors are already in clinical trials for various diseases.

Conflict of Interest The authors have no conflict of interest

Influence of TH-knockout in CD11b+ myeloid cells on bone and immune phenotype of male mice and its effects on stress-induced pathologies during fracture healing

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Introduction Chronic psychosocial stress is a risk factor for impaired fracture healing. Stress-induced synthesis of catecholamines by CD11b + myeloid cells locally in the fracture callus is assumed to contribute to delayed bone healing [1]. It was hypothesized that the knockout of tyrosine hydroxylase in CD11b + myeloid cells, the rate-limiting enzyme of catecholamine synthesis, improves impaired fracture healing in chronically stressed male mice. To analyze that, myeloid cell-specific TH-knockout (KO) mice were generated.

Material and Methods The bone phenotype of CD11b-Cre THflox KO mice was analyzed by µCT and histology. Immunohistochemical staining and FACS-analysis were performed to quantify CD11b + , Ly6G + and F4/80 + immune cells of the bone marrow. To study the influence of TH-knockout on stress-induced pathologies during fracture healing, TH-knockout mice underwent the CSC stress paradigma, received a unilateral femur osteotomy and were sacrificed after 10 days. Histomorphometric analysis, quantification of bone cells and immunohistochemical staining of β2-receptor and endothelial cells were performed with fractured femora.

Results TH-knockout mice showed no changes in femoral length, growth-plate thickness and bone cells. Trabecular bone mineral density was significantly decreased. No changes were observed regarding the analyzed immune cells. During fracture healing, the TH-knockout led to normalization of bone cells and β2-receptor-expression in the callus. However, vascularization and callus composition remained unchanged.

Conclusion The TH-knockout in CD11b + myeloid cells had no influence on bone and immune phenotype under physiological conditions. However, the TH-knockout improved fracture healing in chronically stressed mice through abolishing stress-induced pathologies.

Conflict of Interest None.

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Applications and limitations of finite element method, multibody simulation, and hybrid modeling in bone biomechanics

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Introduction Computational modeling of the musculoskeletal system is an efficient, non-invasive method for studying the mechanical behavior of tissues.

Material and Methods This review presents four computational methods: Finite element structure analysis (FESA), finite element structure synthesis (FESS), multibody simulation, and hybrid models. Their applications and limits are discussed.

Results FESA is an inductive method with mesh size dependent results. It provides spatial results for strain and stress in solids or velocity and pressure in fluids. However, FESA of complex geometries or including non-linear contacts or dynamic problems is limited by time-consuming manual mesh repairs and high computational time. In FESS, the implementation of mechanobiological rules allows to deduce biological reactions to mechanical conditions during fracture healing, growth, and remodeling [1]. Muscle activation patterns and joint movements are common applications for multibody simulations. Using rigid bodies, the main area of application is in the context of dynamics, but analyses of internal loads are limited. Combining finite element and multibody simulations, stress and movement can be calculated to simultaneously optimize muscle forces and morphology [2]. Hybrid finite element multibody models combine rigid and elastic bodies to simulate active and passive structures in a single model while reducing computational time compared to iterative coupled FE-multibody models [3]. Computational models are highly sensitive to changes in geometry, materials, loading, and mechanobiological thresholds. Consequently, a comprehensive validation requires the development of dedicated approaches.

Conclusion Computational models support a better understanding of biomechanical principles. Further collaborative research is needed for the transition to clinical applications.

Conflict of Interest No conflict of interest to declare.

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The osteosclerotic phenotype of Fra1 transgenic mice is not reversed by Lrp5 or Wnt1 deletion in osteoblasts

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Introduction Ubiquitous overexpression of *Fra1* (*Fosl1*), a Fos member of the AP-1 transcription factor family, induces osteosclerosis due to an increased bone formation [1]. The exact mechanism leading to the increased bone mass has not yet been described. A similar phenotype, however, was shown for Lrp5 gain-of-function mutants [2] and osteoblast-specific inducible *Wnt1* transgenic mice (*Wnt1*Tg) [3]. Additionally, *Fra1*Tg mice show increased Wnt1 serum levels. Therefore, we analyzed if the osteosclerosis of *Fra1*Tg mice can be rescued by deletion of Lrp5 or Wnt1 in osteoblasts.

Material and Methods *Fra1* transgenic mice with a deletion of *Wnt1* in osteoblasts by *Runx2*-cre, as well as *Fra1* transgenic *Lrp5* deficient mice were generated. The bone phenotype was evaluated by static and dynamic histomorphometry.

Results Osteoblast-specific *Wnt1* deletion in *Fra1*Tg mice (*Fra1*Tg;*Wnt1*$^{fl/fl}$;*Runx2*-cre) did not reverse the increased cortical and trabecular bone mass of *Fra1*Tg mice. Furthermore, *Fra1*Tg;*Wnt1*$^{fl/fl}$;*Runx2*-cre mice developed lipodystrophy, fibrosis of lung and liver and splenomegaly, that are known to be caused by Fra1 overexpression, suggesting that the phenotype of *Fra1*Tg mice is independent of Wnt1 expression in osteoblasts.

Similarly, analyses of *Lrp5*-deficient *Fra1*-transgenic mice (*Fra1*Tg;*Lrp5*$^{-/-}$) showed that Fra1-induced osteosclerosis was not affected by Lrp5 inactivation, and additionally, that *Fra1*Tg;*Lrp5*$^{-/-}$ mice show a lipodystrophic phenotype, fibrosis and splenomegaly. Therefore, Fra1 and Lrp5 seem to act via independent bone-anabolic pathways.

Conclusion These results show that the progressive osteosclerosis of *Fra1*Tg mice is independent of Wnt1 expression in osteoblasts and of Lrp5 expression.

Conflict of Interest None.

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Inhibition of Foxo3 during myogenic differentiation

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Introduction Patients suffering from disease-related (secondary) sarcopenia have been associated with an enhanced level of the transcription factor *Foxo3* in skeletal muscle [1–3].

Sarcopenia is a progressive and generalized muscle disorder characterized by a decline in muscle mass and strength [4]. If left untreated, it can lead to increased falls, fractures [5, 6], mortality [7], reduced quality of life [8], as well as increased hospitalization rates and cost of care [9].

Foxo3 is one of several transcription factors of the highly conserved Forkhead-Box-Protein family [10]. As a downstream target of the *PI3K*/*AKT* pathway [11], *Foxo3* plays an important role in protein turnover and muscle wasting [12]. *Foxo3* therefore could pose to be a potential target of treatment for secondary sarcopenia.

The focus of this study is to reduce the *Foxo3*-expression in murine myoblasts *in vitro* and to analyze changes in myogenic differentiation.

Material and Methods Foxo3 knockdown was achieved via single and double lipofection of Foxo3-siRNA. Subsequently, we differentiated myoblasts to myotubes. Length and width of myotubes were measured via light microscopy. Foxo3 mRNA analysis was performed by RT-qPCR.

Results A Foxo3-mRNA knockdown has been confirmed 1-8 days after siRNA transfection with additional decrease of Atrogin1-mRNA. Morphologically, treated myotubes show an increase in length and width. Live/dead cell viability assay showed no significant difference between treated and untreated cells.

Conclusion We were able to reach a significant *Foxo3*-mRNA knockdown of up to 82 % after lipofection. As downstream member of the *Foxo3* pathway, a 32 % decrease of *Atrogin1*-mRNA was measured after lipofection. Morphologically, *Foxo3*-inhibited myotubes showed an increase in length and width.

Conflict of Interest The authors declare no conflict of interest.

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β2-adrenoceptor deficiency in experimental osteoarthritis leads to exacerbation of subchondral bone changes without affecting cartilage and synovium

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Introduction Recent studies demonstrated a contribution of the sympathetic nervous system and its major neurotransmitter norepinephrine (NE) to osteoarthritis (OA) pathogenesis. The effects of NE are predominantly catabolic in cartilage and subchondral bone, while mainly anti-inflammatory in the synovial tissue [1, 2]. Several adrenoceptor (AR) subtypes are expressed in different joint tissues [3] but the current literature suggests that the β2-AR plays a crucial role during OA pathogenesis [4–6]. Therefore, we examined the progression of surgically induced OA in β2-AR-deficient (Adrb2$^{-/-}$) mice.

Material and Methods OA was induced by destabilization of the medial meniscus (DMM) in wild type (WT) and Adrb2$^{-/-}$ mice. 8 weeks after DMM or sham surgery, body weight was determined. Bone parameters such as subchondral bone plate (SCBP) thickness, bone volume over total volume (BV/TV), trabecular thickness (Tb.Th), trabecular space (Tb.Sp) were analyzed by micro-computed tomography (μCT), and the severity of OA was assessed by histological scoring (OARSI and synovitis grade).

Results Adrb2$^{-/-}$ mice had significantly higher body weight compared to WT mice (Adrb2$^{-/-}$ 33.94 ± 0.87 g, WT 28.67 ± 0.18 g; p = < 0.0001). In sham animals, there were no significant differences in bone parameters between WT and Adrb2$^{-/-}$. In contrast, DMM-operated Adrb2$^{-/-}$ mice displayed significantly increased subchondral bone plate thickness (WT DMM 107.7 ± 3.1 μm, Adrb2$^{-/-}$ DMM 160.01 ± 14.27 μm; p = 0.0098), BV/TV (WT DMM 0.563 ± 0.029, Adrb2$^{-/-}$ DMM 0.697 ± 0.026; p = 0.014) and Tb.Th (WT DMM 0.08 ± 0.008 mm, Adrb2$^{-/-}$ DMM 0.506 ± 0.084 mm; p = 0.0055) in the medial epiphysis. At the same time, Tb.Sp was significantly decreased (WT DMM 0.054 ± 0.002 mm, Adrb2$^{-/-}$ 0.022 ± 0.005 mm; p < 0.0001). WT and Adrb2$^{-/-}$ mice subjected to DMM developed comparable changes in cartilage degeneration and synovial inflammation (mean OARSI score: WT DMM 3.00 ± 0.47, Adrb2$^{-/-}$ DMM 2.85 ± 0.57; mean synovitis score WT DMM 2.86 ± 0.26, Adrb2$^{-/-}$ 2.75 ± 0.25).

Conclusion Subchondral sclerosis is a hallmark of OA. We observed an increased bone mass in Adrb2$^{-/-}$ DMM versus WT DMM mice. This is consistent with earlier studies reporting an anti-osteogenic effect of β2-AR. However, we did not detect any difference between bone parameters of sham-operated WT and Adrb2$^{-/-}$ animals suggesting that increased bone mass in Adrb2$^{-/-}$ DMM mice is attributed to a synergistic effect of β2-AR deficiency and OA. The elevated body weight in Adrb2$^{-/-}$ mice might result in increased mechanical joint loading. However, this was not sufficient to induce a more pronounced cartilage degeneration and synovitis than in WT mice. Taken together, β2-AR plays an important role in OA-related bone changes and could thus be an attractive target for novel therapeutic treatments.

Conflict of Interest The authors declare that there are no conflicts of interest.

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Mechanical stress leads to morphology change of MC3T3 osteoblastic cells

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Introduction The different cell types in the musculoskeletal system experience mechanical stress. [1] To simulate the in vivo conditions in in vitro conditions cell stretch systems are available to apply mechanical stress on cells. [2] Con-

cerning the musculoskeletal system, the conditions for tenocytes are examined well. [1] Osteoblast cultivation, however, is not yet investigated in detail. We will adapt the cell-specific culture-parameters to replicate the typical long-term experiments like mineralization under cell stretching conditions.

Material and Methods We used the FlexCell® FX-600TM to cultivate the osteoblastic cell line MC3T3 and to apply mechanical stress in form of equibiaxial strain with several amplitudes in sine wave with 0.1 Hz. The mineralization was established by alizarin red staining. After immunohistochemistry staining cytoskeletons of the cells were analyzed with the FIJI software (Image J2).

Results The cells had a higher mineralization when they were stretched. The morphology changed and the cells grew insular. Strain exceeding 2.5 % caused cell detachment at long-term culture.

Conclusion We found stretching limits for long-term cultivation regarding time and amplitude. Long-term the MC3T3 cells withstood an over physiological strain of 2.5 %. Higher amplitudes can be recommended just for experiments < 14 days.

Applied mechanical stress led to improved mineralization. This effect subsided with lower stretching amplitudes.

We saw that the cells reacted to even low mechanical stress in a short time by changing their morphology and orientating their stress fibers. Closer analysis showed that within one cell up to 80 % of the stress fibers were aligned.

Conflict of Interest All authors declare that they have no conflict of interest.

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Involvement of tissue non-specific alkaline phosphatase in adipogenic program of bone marrow adipose tissue cells

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Introduction Accumulation of bone marrow adipose tissue (MAT) within bone during life interferes with bone health as well as systemic metabolism. We here investigated the implication of tissue non-specific alkaline phosphatase (TNAP) in MAT-derived cell differentiation.

Methods Bone marrow (BM) samples were obtained from patients undergoing hip surgery and used for bone marrow (BM)-mesenchymal stromal cells (MSC) isolation. BM floating fatty fraction was used for isolation of MAT-cells, while subcutaneous fat from matched donors was used for isolation of peripheral adipose tissue (PAT) cells. Adipogenic differentiation was assessed in absence or presence of the reversible TNAP inhibitor (levamisole hydrochlodride). TNAP expression was estimated by TNAP enzymatic activity assay, and immunofluorescence microscopy combined with mitochondrial staining. Adipogenic marker and *Alpl* gene expression was analyzed by qPCR. Flow cytometry was applied to investigate functional surface markers.

Results MAT and PAT cells displayed stronger adipogenic potential associated with their higher CEBPα and PPARγ gene expression than BM-MSC cells. Enzymatic activity, protein and gene expression suggested higher basal expression of TNAP in MAT and PAT cells. Increase of TNAP activity and *Alpl* mRNA level during adipogenesis was observed at the higher extent in MAT and PAT cells. Inhibition of TNAP activity, coincided with decreased adipogenesis in all three cell types as well as altered expression of CD36 (receptor involved in fatty acid traffic) and CD142 (coagulation factor III).

Conclusions Results suggest that TNAP might play an important role in adipogenic differentiation, and further investigations will clarify mechanisms of TNAP-regulated adipogenesis in BM cells.

Conflict of interest There are no conflicts of interest.

Magnesium phosphate granules support bone regeneration in a large animal model

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Introduction Magnesium phosphate (MgP) minerals have comparable characteristics to calcium phosphate-based bone substitutes, but degrade more rapidly under physiological conditions [1–3]. The current study aimed to evaluate the bone regeneration capacity of MgP granules in a mechanically unloaded, critical-size defect model in the trabecular bone of sheep.

Material and Methods The animal experiment was approved by Regierungspräsidium Tübingen (Nr. 1451). 14 adult female Merino sheep were used in this study. Each animal received two different types of MgP granules, struvite and K-struvite, that were implanted bilaterally into critical-sized bone defects in the medial femoral condyle. Two (n = 7) and four (n = 7) months after surgery, the degradation of the granules and new bone formation were assessed by micro-computed tomography (μ-CT), histological and biomechanical analysis.

Results Macroscopically, no signs of inflammation were observed. The struvite granules showed a continuous degradation with simultaneous new bone formation. In contrast, the K-struvite granules were almost completely resorbed and replaced by newly formed bone already after two months of implantation. Histomorphometry revealed that the rapid degradation of K-struvite was associated with a significantly higher amount of soft tissue formation. The complete osseointegration of the struvite granules resulted in a significant increase in stiffness within four months.

Conclusion This study demonstrated the biocompatibility of the implanted materials. K-struvite granules appear to be promising for the application in mechanically unloaded situations and small bone defects, where a rapid degradation is desirable. Struvite granules may be more useful for the treatment of larger defects because of their slower degradation.

Conflict of Interest No conflict of interest.

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Cell-cell communication between chondrocytes and osteoblasts through miR-221-3p loaded extracellular vesicles

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Introduction Osteoarthritis (OA) is considered as a whole joint disease. The close physical association between subchondral bone and cartilage suggests the existence of biochemical and molecular crosstalk across the bone-cartilage interface, which may even be elevated in OA [1, 2]. miR-221-3p was demonstrated to be mechanosensitive in cartilage chondrocytes and extracellular vesicles (EVs) have been deeply researched for a specific role in cell-cell communication [3–5]. The present study is to investigate the communication between chondrocytes and osteoblasts through miR-221-3p transferred via EVs.

Material and Methods Chondrocytes and osteoblasts were isolated from newborn rats. EVs were isolated by differential ultracentrifugation. Nanoparticle tracking analysis, western blot analysis and transmission electron microscopy were used to identify EVs. Chondrocytes and osteoblasts were cocultured in transwell and qRT-PCR was used to assess their communication. Osteoblasts were treated with EVs isolated from chondrocytes and results were evaluated by histochemistry methods and qRT-PCR.

Results Collagen II immunofluorescence was positive in chondrocytes and mineralized nodule formation of osteoblasts was illustrated by Von Kossa and Alizarin red staining. miR-221-3p was transferred by EVs from chondrocytes to osteoblasts in our coculture transwell approach. Furthermore, qRT-PCR and Alizarin red staining demonstrated a decreased osteogenic capacity of osteoblasts treated with miR-221-3p loaded EVs.

Conclusion This study provides a novel perspective on the mechanotransductive effect of miR-221-3p between chondrocytes and osteoblasts' communication through EVs. These results build the basis to modulate bone-cartilage remodeling and communication in the future.

Conflict of Interest No potential conflict of interest was reported by the authors.

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Platelet-rich plasma factors enhance bone marrow stromal cell motility and survival by activating AKT signaling

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Introduction Early phase of bone healing is coupled with hematoma formation which precedes regenerative processes. To investigate hematoma components, we explored the effects of platelet-rich plasma (PRP)-derived factors on MSC survival and motility.

Material and Methods MSCs were isolated from bone marrow of hip arthroplasty patients. PRP was collected from thrombocyte concentrates and hydrogel formation was induced by thrombin. Conditioned media (CM) was collected after incubating activated PRP and pure fibrin (FBR) hydrogels for 24 h. MSCs were exposed to 10 % CM and viability was investigated evaluating metabolic activity and ATP content. Scratch assays were performed and MSCs were co-cultured with endothelial cells (HUVEC-GFP) in transwells to test cellular motility and migration. Mitochondria and F-actin were estimated by fluorescence imaging. Cell cycle and apoptosis were assessed by flow cytometry. Western blot and qPCR were used for protein and gene expression analysis.

Results PRP-CM showed moderate effects on viability and ATP content, without influence on cell cycle and apoptosis. MSC motility and migration were stimulated in presence of PRP-CM and inhibited in groups with autophagy inhibitors and activators compared to the control group. PRP-CM induced, whereas FBR-derived CM decreased phosphorylation of protein kinase B (pAKT) in MSCs in time-dependent manner. PRP-CM upregulated gene expression of anti–apoptotic (*Bcl-2*), autophagy markers (*ATG7*) and osteoblastic factor (*RunX2*).

Conclusion Our findings indicate that changes in MSC mobility mediated by PRP-CM might be controlled by AKT signaling pathway. Future studies will show how these effects can be used for control and enhance of bone tissue regeneration.

Conflict of Interest There are no conflicts of interest.

3D tissue engineering with a novel bioreactor

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Introduction 3D cultivation provides a more realistic model of human tissue in comparison to traditional 2D cultivation [1, 2]. Various 3D cell culture systems have been developed so far to study the three dimensional interaction between cells and the extracellular matrix surrounding them and to later create artificial tissues [3, 4]. 3D culture techniques with the possibility of constant supply of fresh nutrients are essential, but not yet broadly established.

Material and Methods We designed a bioreactor with the open source software *blender* for 3D printing (*Ultimaker 3)*. The reactor is made of non-toxic, biocompatible polylactic acid and can be connected to a perfusion system (*Ibidi Pump System)* for medium flow.

For 3D culture we used polyethylene glycol diacrylate (PEGDA) hydrogels of different molecular weights which are cured by UV light exposure.

In those hydrogels we cultured SCP-1 cells and primary human bone marrow mesenchymal stem cells which we isolated from human femoral heads.

Results Cells survived three weeks of cultivation in our bioreactor shown by live-dead staining and fluorescence microscopy. Further, the bioreactor is suitable for long time cultivation since the impermeableness, perfusion channel and fluid flow maintained after prolonged cultivation.

Conclusion We established a novel bioreactor for 3D tissue engineering where an artificial vessel provides a customizable, constant medium flow. We compared different hydrogels and found PEGDA to be the most fitting. With this novel system further research can be done to investigate the cell-matrix interaction and to pursue biocompatible, size and shape adjustable artificial engineered tissue.

Conflict of Interest The authors declare no conflict of interest.

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Assessment of bone matrix composition in teleost fish and humans using Raman spectroscopy

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Introduction Bony fish (i.e. teleosts) are emerging small-sized animal models to investigate bone quality, metabolism, and disease pathomechanisms. In particular, species as the swordfish (Xiphias gladius) [1] and killifish (Nothobranchius furzeri) [2] have been recently introduced to the musculoskeletal disciplines because of their peculiar hard tissue biology. We carried out a comprehensive ex vivo analysis of bone tissue to compare the hard tissue properties of the swordfish rostrum, and the killifish vertebral bone with human bone tissue. This macromolecular study of the tissue composition reveals species-dependent similarities and differences in tissue composition.

Material & Methods Bone matrix composition of killifish (n = 3), swordfish rostrum (n = 3) and humans (n = 6) were analyzed using Raman spectroscopy. Spectra were collected with the scan range from 400 to 1800 cm-1 . Twenty bone spectra/sample were baseline-subtracted and averaged. Mineral-to-matrix ratios (MMR) were calculated: v1PO4 (930–980 cm-1)/amide I (1620–1700 cm1), v1PO4/amide III (1215–1300 cm-1) and carbonate-to-phosphate ratio (CPR) CO3(1050–1100 cm-1)/v1PO4 [3].

Results Both MMRs did not differ between swordfish and human samples ($p > 0.05$ for both) but were significantly different between killifish and the other two groups (pswordfish0.05 for both).

Conclusion Differences in mineral composition in the studied species were evident. Low CPR in both fish was found in comparison to human bone. The low CPR may be linked to differences in mineral metabolism required in the aquatic environments. Generally, our results point towards the presence of the same macromolecular components including phosphate, carbonate, amide I, and amide III in all bone types. Further studies will help to better understand the width of hard tissue properties contributing to individual bone quality characteristics across species.

Conflict of Interest The authors declare no conflict of interest.

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Col22a1 deficiency leads to trabecular bone loss in mice

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Introduction In an unbiased screening approach we identified *Col22a1*, encoding Collagen type XXII, as one of the most strongly induced genes during *in vitro* osteoblast differentiation [1]. Furthermore, expression of *Col22a1* was determined to be highly specific for bone tissue. *Col22a1* belongs to the FACIT family of collagens that does not form fibrils and has previously been identified mainly in tissue junctions [2]. Therefore, we aimed to explore its function in the skeleton.

Material and Methods We have performed extensive skeletal phenotyping in a *Col22a1*-deficiency mouse model via µCT-analysis and histomorphometry of 6 to 24 weeks old animals as well as *ex vivo* cell culture.

Results Analysis of the skeleton from *Col22a1*-deficient mice revealed a specific decrease of trabecular bone mass (BV/TV) in the spine and femur while the cortical parameters were not affected. Cellular histomorphometry surprisingly indicated an increased osteoclast number as the underlying cellular mechanisms while cell culture experiments demonstrated the lack of a cell autonomous phenotype.

Conclusion In conclusion, we were able to demonstrate for the first time that the FACIT Collagen type XXII is required for the formation of proper trabecular microarchitecture.

Conflict of Interest None.

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Index