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ABSTRACTS

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The scientific merit of the abstract
Suitable sample size
Adherence to instructions
Originality of work

The FishBone Workshop was held on May 12, 2017 in conjunction with the ECTS Annual Congress in Salzburg. The abstracts were reviewed by the organizers of the FishBone Workshop and publication was approved by ECTS.

P-BDEV-5

CHRONIC PSYCHOSOCIAL STRESS DISTURBS ENDOCHONDRAL OSSIFICATION

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Objectives: Clinical investigations showed that patients suffering from depression display a higher incidence for osteoporosis [Calarge 2014]. Confirming, mice subjected to chronic mild stress, a model for depressive-like behavior, displayed decreased bone mass, probably due to increased levels of glucocorticoids (GCs) [Azuma 2015]. In contrast to chronic mild stress, chronic psychosocial stress (CPS) does not induce increased GC levels [Langgartner 2015], thus likely affecting bone differentially. Therefore, we investigated the effects of CPS on bone using the murine subordinate colony housing (CSC) model.

Methods: 7-weeks-old male C57BL/6-mice were subjected to CSC for 19 days [Reber 2007]. CSC-mice were housed in groups of four together with a dominant male CD1-mouse in order to induce chronic subordination. Single-housed (SHC)-mice were used as control. On day 20, femurs were subjected to µCT-analysis, histomorphometry and immunohistochemistry. Blood was collected for corticosterone analysis. Adrenal glands were analyzed by western-blotting. n= 8/group. Student's t-test, *p≤0.05.

Results: Cortical and trabecular thickness as well as BMD were significantly increased after CSC, whereas trabecular number was reduced. Number of osteoblasts and osteoclasts did not differ between the groups. Femur and tibia length were significantly reduced (SHC: 16.0 mm/18.8 mm, CSC: 15.6mm*/17.8mm*), whereas growth plate (GP) thickness was significantly increased (SHC: 119 µm, CSC: 147µm*) due to an accumulation of hypertrophic chondrocytes. Runx2 expression was decreased in the hypertrophic and calcification zone of the GP, indicating reduced cartilage-to-bone transition during endochondral ossification. GP tyrosine hydroxylase (TH)-protein expression was strongly upregulated in CSC mice, suggesting an involvement of the sympathetic nervous system. Underlining this, adrenal weight and TH expression were significantly increased after CSC, whereas plasma corticosterone levels did not differ between the groups.

Summary & Conclusion: Since noradrenaline was shown to decrease expression of chondrocytic terminal differentiation markers like Runx2 and MMPs [Du 2014], we hypothesize that disturbed endochondral ossification after CSC might be associated with increased sympathetic activation.

Disclosure: No significant relationships.

Keywords: bone metabolism, noradrenaline, endochondral ossification, psychosocial stress, sympathetic nervous system

P-BDEV-6

This abstract has been withdrawn.

P-BDEV-7

THE IMPORTANCE OF MITOCHONDRIAL AND RIBOSOMAL GENES IN BONE HEALING

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Objectives: Fracture healing involves a series of consecutive overlapping cellular events. This study aims to unravel the importance of mitochondrial and ribosomal genes during fracture healing process.

Methods: A closed standard mid diaphyseal fracture in the left femur of 8 weeks old C57BL/6N male mice were analyzed at (day= D) D3,D7,D10,D14, D21&D28 post fracture (N=5/time point). Total RNA was isolated to perform whole genome expression profiling. "R" language was used for data normalization and statistical analysis. Differentially expressed genes (DEGs) were obtained using threshold of fold-change (FC) ≥ |2| and p-value≤0.01. Gene ontology analysis was performed using NCBI-DAVID to identify mitochondrial and ribosomal genes. Cytoscape and KEGG were used to perform the regulatory network and pathway analysis. Furthermore, DEGs were mapped with human skeletal disorders.

Results: Despite the importance of biological processes like angiogenesis, ossification, extracellular matrix (ECM), immune response behind the fracture healing process, very little is known about the importance of mitochondrial and ribosomal genes during different stages of healing. This study mainly addresses the DEGs involved in mitochondrial and ribosomal activity. Gene ontology analysis showed the presence of angiogenesis, ossification, ECM, immune response, mitochondrial and ribosomal activity. Mitochondrial and ribosomal genes are crucial for energy metabolism and protein synthesis respectively. Mitochondrial genes were significantly downregulated at all time points. Ribosomal genes were significantly downregulated only at D3 and D7. Genes like 3-Oxoacid CoA—transferase 1 (Oxct1), Nucleophosmin (Npm1), and Electron transfer flavoprotein alpha subunit (Etfa) was significantly downregulated during early stage of fracture healing. Oxct1 is important for ketone body catabolism. Npm1 gene is involved in cell proliferation activity. Etfa gene serves as an electron acceptor during energy metabolism and respiratory electron transport. Currently, the detailed and regulatory role of mitochondrial and ribosomal genes during Wnt and MAPK pathways are being investigated.

Summary & Conclusion: Bone diseases or fractures leading to delayed or nun-union healing are often treated to enhance bone formation. Therefore, biological investigation of gene expression is important to design systemic or local therapeutic agents.

Disclosure: No significant relationships.

Keywords: ribosomal, fracture healing, mouse model, mitochondria

P-BDEV-8

BIOCOMPATIBILITY AND OSTEOCONDUCTIVE PROPERTIES OF A NEW BOVINE BONE GRAFT

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Objectives: Bovine materials are biocompatible and osteoconductive, allowing newly formed bone apposition and partial remodeling process. Synergy Bone Matrix (SBM) (Odontit Implant System) effects on bone healing were compared to those of Bio-Oss (BO) (Geistlich).

Methods: A critical sized bone defect (CZD) was created on both sides of the mandible in rabbits, and filled either with SBM or BO, or

remained unfilled (control). Animals were sacrificed at 4, 8 and 12 weeks. We assessed clinical and biochemically systemic toxicity; lungs, kidney and liver pathology; bone formation and device resorption histomorphometrically; X-rays; and compression and flexural biomechanical tests.

Results: None of the rabbits presented signs of systemic toxicity. Control rabbits developed either fibrosis or adipose tissue independently of time-period; new bone formation or remaining bone substitute amount did not show significant differences between SBM and BO (p=ns). CZD in mandibles filled with both bone grafts exhibited radiopacity compatible with proper healing and gradual replacement of bone grafts; no significant differences between SBM and BO were observed for elastic modulus, shear modulus and compressive strength while control group showed significantly lower values ($p<0.001$).

Summary & Conclusion: Our findings suggest that both biomaterials are similar regarding bone regeneration, osteoconduction and newly formed bone quality. Therefore SBM, the new bone material, can be used for the repairing of bone defects.

Disclosure: No significant relationships.

Keywords: bone regeneration, bovine bone graft, critical sized bone defect, osteoconduction

P-BDEV-9

CRISPR-CAS9 LRP5 KNOCK-OUT ZEBRAFISH AS A MODEL FOR DEVELOPMENTAL DISEASES OF BONE MASS

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Objectives: Lrp5 is a co-receptor in the Wnt-signaling pathway which controls differentiation and proliferation of osteoblasts. In humans lrp5 mutations affect bone mass and cause either osteoporosis or osteopetrosis. Zebrafish lrp5 knockout (KO) may help deciphering the mechanism by which lrp5 affects peak bone mass development. The purpose of our study is to establish a zebrafish model for bone mass diseases and study the effect of lrp5 knock-out on Wnt signaling pathway in early life.

Methods: CRISPR-Cas9 was used to create lrp5 exon 2 nonsense mutation (p.Ala53fs), resulting in lrp5 knockout. Embryos (F0) at 1-cell stage were injected with Cas9 mRNA and lrp5 gRNA and were grown to adulthood. F0 carrying lrp5 nonsense mutation was crossed to WT and F2 progeny was obtained. At 8 and 9 days post fertilization F2 embryos were stained with calcein and visualized under fluorescent microscope. The number of mineralized vertebrae, adjusted for body length, was compared between wild type (WT), heterozygote ($lrp5^{-/+}$) and homozygote (lrp5-KO) zebrafish.

Results: We found a trend showing a decrease in the number of mineralized vertebrae and the body length in $lrp5^{-/-}$ compared with $lrp5^{+/+}$ WT. Moreover we found that the homozygote $lrp5^{-/-}$mortality rate was significantly higher than the heterozygote and WT $lrp5^{+/+}$ between the ages of 14 days to 3 months post fertilization.

Summary & Conclusion: Our study established an lrp5 knockout zebrafish model and showed that the lrp5 gene has a role in the early stages of zebrafish vertebrae ossification and body growth. This study indicates that the lrp5 gene is essential for the zebrafish development and that its dysfunction leads to high mortality rate prior to reaching adulthood.

Disclosure: No significant relationships.

Keywords: CRISPR-Cas9, Lrp5, Wnt-signaling pathway, zebrafish model, bone mass diseases

P-BDEV-10

STRUCTURAL CHANGES OF BONE MATRIX AND EXTRACELLULAR MATRIX IN YOUNG AND OLD RATS

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Objectives: Aging related bone loss is described by a dysregulated osteoblast activity, leading to a low-turnover phenotype, reduced bone mass and low bone mineral content. Juvenile bone has an increased matrix formation and mineralization with Endochondral ossification involved in natural growth. Ultrastructure characterization of bone matrix to quench alterations between juvenile and old bone require the optimization of bone preparation prior to high-resolution electron and correlative light microscopy. Therefore, we have developed a new preparation protocol using microwave assisted fixation to attain tissue properties for best comparison.

Methods: Juvenile rats 2 weeks of age and Aged rats 15 month old were compared using microwave assisted chemical fixation for histomorphometrical preparation to achieve a close-to-native preservation of bone tissue. Immunohistochemistry examination of Type—one collagen (Col1) was examined to study collagen fibrils orientation. Investigative histological stains were applied to study mineralization rate and trabecular bone properties using Von Kossa/Van Gieson staining. Furthermore, ultrastructural component of osteocyte and their canaliculi in young and old rat femur were studied using transmission electron microscopy. The organic matrix structure was investigated using computational segmentation of collagen fibrils.

Results: Aged bone showed less compact structure and inhomogeneous Col1 distribution. Osteocytes of aged bone showed ultrastructural changes compared to osteocytes of young bone. Silver nitrate staining demonstrated lesser osteocyte population in aged bone, reduced canaliculi numbers, higher frequent of spherical shaped cells, irregular alignment of cells along matrix. Abnormal pattern of mineral distribution in von Kossa/van Giesson. Collagen fibrils appeared randomly oriented, thinner, and shorter when compared to young bone.

Summary & Conclusion: The inferior bone properties of aged bone might be used to explain the changes in quality of bone functionality. A conclusive structural description and evaluation of age related alteration in bone matrix is in process with correlation to mechanotransduction and ERK regulation and blood vessel formation.

Disclosure: No significant relationships.

Keywords: rates, mineralization rat, osteocytes, bone, aging

P-BDEV-11

MILK ACTIVATES TGF-β SIGNALING: POSSIBLE IMPLICATION IN CELLS OF THE ORAL CAVITY

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Objectives: Transforming growth factor β (TGF-β) in milk is supposed to maintain intestinal mucosal homeostasis of the neonate. The impact of TGF-β in milk on cells of the periodontium, however,