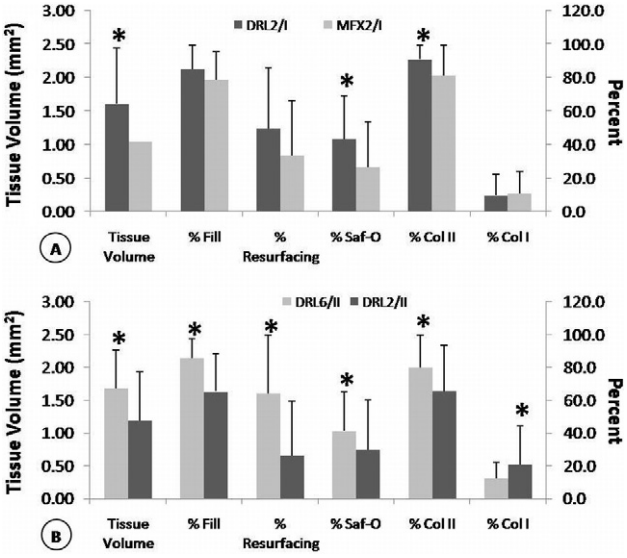

Figure 3. Effects of bone marrow stimulating procedure by microfracture (MFX2 holes vs drilling (DRL2 holes) to the same depth of 2 mm (A) and of the depth of drill holes (6 mm DRL6 vs 2 mm DRL2) (B) on tissue repair in cartilage defects from rabbit trochleas 90 days post-operatively. *$P<0.05$.

collagen type II and less collagen type I in the repair matrix ($P<0.04$ for all) (Fig. 3).

Conclusions: Surgical techniques affect the patterns and connectivity of subchondral bone marrow channels, thus influencing cartilage repair outcomes. Bone marrow stimulation by DRL provided free channels to marrow stroma and led to significantly better cartilage repair than MFX at 3 months. Compared to shallow perforation, deep DRL with increased access to marrow compartments produced more effective hyaline-like cartilage repair in rabbits. These findings suggest a surgical technique that cleanly removes bone and bone fragments and provides free access to marrow may be superior to MFX as a bone marrow stimulation technique for cartilage repair.

Moderated Poster Session 2

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THE ROLE OF AKT1 IN TERMINAL STAGES OF ENDOCHONDRAL BONE FORMATION: ANGIOGENESIS AND OSSIFICATION

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Purpose: Longitudinal bone growth is the result of endochondral bone formation which takes place in the growth plate. The rate of chondrocyte proliferation and hypertrophy, vascular invasion with the formation of primary ossification centers and cartilage replacement by bone tissue are all important processes required for normal growth. We have shown a role for the PI3K signaling pathway in chondrocyte hypertrophy and bone growth in tibia explant cultures. In this current study we aimed to investigate the role of Akt1, an important target of PI3K, in endochondral ossification.

Methods: Mouse long bones were fixed in formaldehyde, paraffin embedded and sectioned. Different staining methods were applied: Safranin O/Fast green for cartilage visualization and TRAP stain for osteoclast activity. Immunohistochemistry was also performed using antibodies against vascular endothelial growth factor (VEGF) and matrix metalloproteinase 14 (MMP-14) in eleven day-old Akt1 KO and control long bones. Tibiae isolated from E15.5 mice were cultured for three weeks in the presence of a PI3K inhibitor (LY294002) or vehicle control. These bones were measures at the beginning and at the end of the time course. MicroCT analysis was performed in seven day- and one year-old Akt1 KO and control mice. Bone mineral density (BMD) and bone mineral content (BMC) were analyzed in the proximal tibia and in the 5th lumbar vertebrae of one year-old Akt1 mice.

Results: Akt1 KO mice showed reduced size compared to their littermates throughout life, but the largest difference in body size was observed around one week of age. Focusing on this specific developmental stage, we discovered delayed secondary ossification in the long bones of Akt1 KO mice. A delay in formation of a structure resembling a secondary ossification center (SOC) was also seen in tibia organ cultures treated with LY294002. The expression of MMP-14, the main protease responsible for development of secondary ossification centers, was decreased in the epiphysis of Akt1 KO mice, possibly explaining the delay in SOC seen in the Akt1 KO mice. BMD and BMC were found to be decreased in one year-old Akt1 KO mice, suggesting that the original delay in ossification affects bone quality in older animals.

Conclusions: We show a novel role for Akt1 protein kinase in the formation of long-bone SOC. The reduction in MMP-14 protein levels in the Akt1 KO mouse tissues suggested a regulatory mechanism possibly responsible for this delay in skeletal development.

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DISTINCT TRANSCRIPTIONAL CONTROL OF CHONDROCYTE HYPERTROPHY AND CARTILAGE DEGRADATION BY C/EBP-BETA AND RUNX2 DURING endochondral ossification

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Purpose: Chondrocyte hypertrophy and cartilage degradation, characterized by expressions of type X collagen (COL10) and matrix metalloproteinase 13 (MMP13), respectively, are sequential and crucial steps in endochondral ossification during skeletal growth and osteoarthritis (OA) progression. This study investigated the role of CCAAT/enhancer-binding protein β (C/EBPβ) in chondrocytes and its interaction with Runx2 during the endochondral ossification.

Methods: To know the physiological functions of C/EBPβ and Runx2, we compared the skeletal phenotypes of the homozygous (–/–) or heterozygous (+/–) deficient mice with the respective wild-type littermates by Alcian blue, Alizarin red and von Kossa stainings, BrdU labeling, and immunostainings of COL10 and MMP13. After an experimental OA model was created surgically by inducing instability in the mouse knee joints, the articular cartilage underwent histological analyses as above and the cartilage destruction was quantified by the OARSI histopathology grading. For the functional analyses, we established stable lines of human chondrogenic SW1353 cells with retroviral transfection of C/EBPβ, Runx2, or both of them. Cell proliferation was assessed by CCK-8 assay. Chondrocyte differentiation was determined by Alcian blue and Alizarin red stainings, as well as expressions of COL10 and MMP-13 by real-time RT-PCR. Promoter activities of COL10 and MMP13 genes were analyzed by luciferase assays in SW1353 cells transfected with reporter constructs containing the respective promoter fragments, and the core responsive regions were determined by the deletion, mutagenesis, and tandem-repeat analyses of the constructs.

Results: C/EBPβ–/– mice exhibited dwarfism from embryonic stages with delayed chondrocyte hypertrophy and decreased