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significantly higher than for NWB pain and "any" dAB, respectively (Fisher Z).

Conclusions: In this cross-sectional study we demonstrated a significant relationship between dAB and pain frequency. The relationship of dABs with WB pain intensity appeared to be somewhat (but not significantly) stronger than for NWB pain, and the relationship for "central" femorotibial dABs somewhat (but not significantly) stronger than of "any" dAB.

062

BONE-MARROW STIMULATION BY DRILLING VERSUS MICROFRACTURE LEADS TO BETTER CARTILAGE REPAIR IN RABBITS

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Purpose: Microfracture (MFX) and drilling (DRL) are widely practiced surgical procedures for bone marrow stimulation cartilage repair, but they have inherent mechanical differences and have not been systematically compared for a desired cartilage repair outcome. This study compared subchondral characteristics and cartilage repair outcomes following MFX versus DRL, and examined the effect of hole depth in a rabbit model.

Methods: Trochlear cartilage defects were prepared bilaterally in 24 skeletally mature rabbits. Perforations were made into subchondral bone using MFX or DRL techniques. MFX holes were made to a depth of 2 mm (MFX2) and drill holes made to either 2 mm or 6 mm depth under cooled irrigation (referred to as DRL2 and DRL6, respectively). Animals were sacrificed 1, 14, 21 and 90 days postoperatively, and defects assessed by micro-CT, histology, immunohistochemistry and histomorphometry.

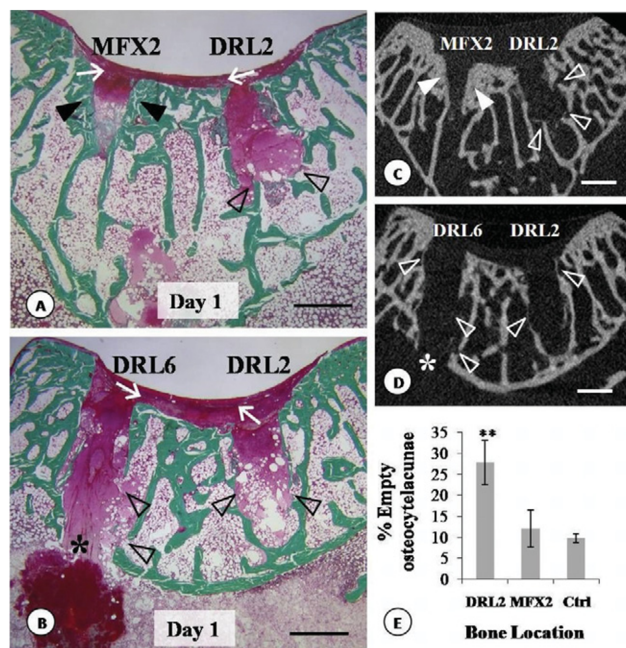

Figure 1. Goldner's Trichrome stained MMA sections (A, B) and vertical micro-CT images (C, D) of cartilage defects with marrow stimulating 2 mm deep drill holes (DRL2) versus 2 mm deep microfracture holes (MFX2) (A, C) in the distal location and 2 mm deep DRL2 versus 6 mm deep DRL6 drill holes (B, D) in the proximal location of the trochlea of rabbits sacrificed 1 day post-operatively. Arrows point to the blood clots covering the defects. Solid arrowheads show the dense and crushed bone around MFX2 holes and empty arrowheads point to marrow openings in DRL holes. * indicates penetration of epiphysial scars and creation of large hematoma by DRL6 holes. Scale bars – 1 mm. (F) shows the percent of osteocystes with empty lacunae in bone adjacent to and 1–2 mm away from holes in control regions in rabbit trochlea at 1 day post-operation. Data are expressed as mean ± sd (n - 4). ***P*<0.05 for the paired Student *t*-test.

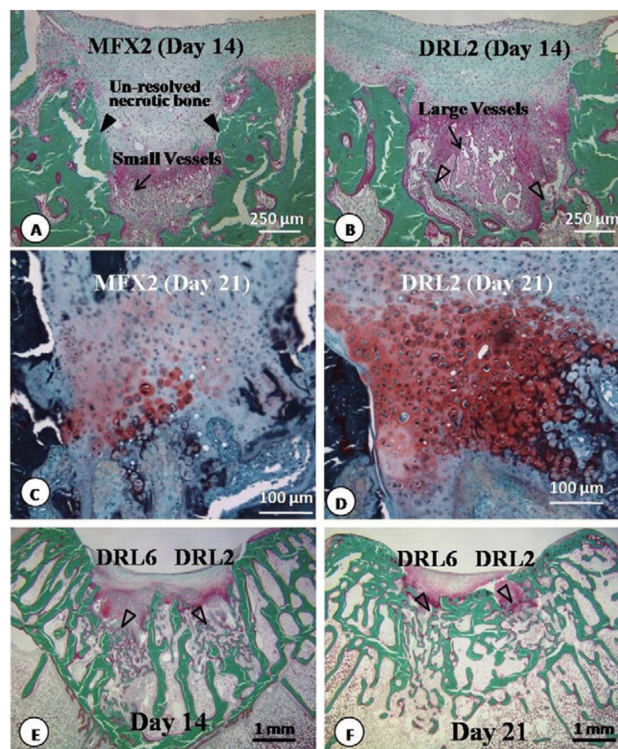

Figure 2. Repair of cartilage defects with microfractured (A, C) and drill (B, D, E and F) holes in rabbit trochleas at day 14 (A, B and E) and day 21 (C, D and F) post-opration. Arrows point to new vessels formed in holes. Solid arrowheads show the presence of un-resolved necrotic bone around MFX2 holes, and empty arrowheads point to bone repair in holes. The MMA sections were stained with Goldner's Trichrome (A, B, E and F) and Safranin-O/Fast Green (C, D).

Results: MFX induced acute crushing and compaction of bone, leaving dense bone around MFX2 holes (Fig. 1A, C) which essentially blocked connection with bone marrow. DRL, however, removed bone and debris from holes to provide access channels to marrow stroma (Fig. 1A–D). In contrast to generally accepted dogma, significantly more empty osteocyte lacunae (bone necrosis) were detected in bone lining the MFX holes, compared to the DRL holes generated under cooled irrigation where no apparent heat necrosis was seen (Fig. 1E). Deeper DRL holes at 6 mm penetrated the epiphysial scar (the closed growth plate) in rabbits and produced greater subchondral hematoma with increased access to deep marrow cavity (Fig. 1B, D).

At Day 14 and Day 21, greater bone repair and a more robust angiogenic and chondrogenic response were seen in DRL vs. MFX holes. Bone repair attained similar heights in DRL6 and DRL2 despite initial greater bone removal in deep holes (Fig. 2).

Results at 90 days revealed that DRL produced more tissue repair than MFX (1.6±0.84 mm^2 vs. 1.0±0.67 mm^2, *P*=0.007), and had a statistically significant increase in the hyaline character vs. MFX as indicated by percent of tissue repair positive for Safranin-O (43.3±25.85% vs. 26.7±26.85%, *P*=0.015) and collagen type II staining (91.0±8.28% vs. 81.2±18.24%, *P*=0.009). Compared to shallow perforation, deep DRL stimulated more effective cartilage repair with significantly greater tissue volume, defect fill and resurfacing (*P*<0.01 for all). The repair in DRL6 was also more hyaline than in DRL2 as judged by significantly more proteoglycan, more

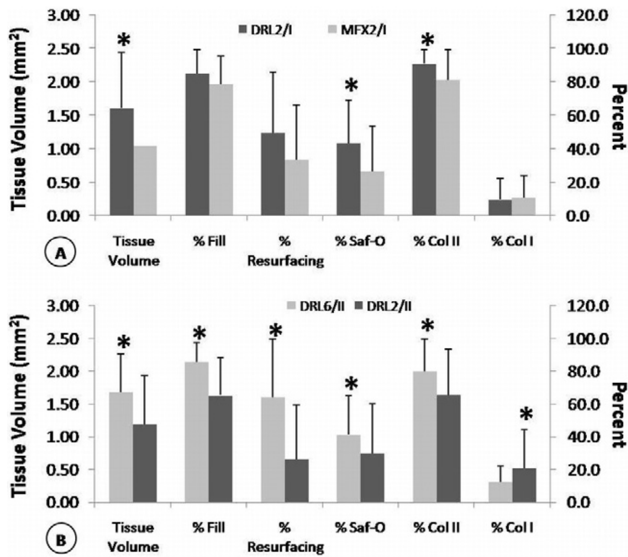

Figure 3. Effects of bone marrow stimulating procedure by microfracture (MFX2 holes vs drilling (DRL2 holes) to the same depth of 2 mm (A) and of the depth of drill holes (6 mm DRL6 vs 2 mm DRL2) (B) on tissue repair in cartilage defects from rabbit trochleas 90 days post-operatively. $^{*}P<0.05$.

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.

064

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