**M043**

**BrDU Tracking of Preosteoblasts in Formalin Fixed, Decalcified Regenerating Bone.** C.S. Wahl*, J. Arason*, R. A. Scanne*, C.K. Lumpkin*, A.A. Arkansas Children's Hospital Research Institute, Little Rock, AR, USA, 2Departments of Orthopedics and Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Distraction osteogenesis (DO) is a limb lengthening procedure that induces rapid bone formation by slowly stretching a surgically introduced fracture at a prescribed rate and rhythm. In order to track the pre-osteoblasts in the distraction gaps of rats, we utilized two types of immunohistochemical markers. The first was an injection of bromodeoxyuridine (BrdU), a thymidine analog, followed by immunohistochemical staining for BrdU positive nuclei. The second was the antibody against proliferating cell nuclear antigen (PCNA). Four male Sprague Dawley rats (350g) underwent standard DO surgery by creating the new ostotomies and inserting the new columns and ostotomized bone segments. The rats were sacrificed at 1, 2, 4, 6, and 12 hours after the operation. The results demonstrated that the distraction tibiae were harvested and stored in neutral buffered formalin (NBF) until processing for histology. A small section of tissue from each rat was also collected as a positive BrdU control. After fixation in NBF, the tibiae were decalcified in 5% formic acid, dehydrated, and embedded in paraffin. Serial sections were then stained with either a mouse monoclonal antibody against PCNA (PC-10), a monoclonal antibody against BrdU or a normal mouse IgG for negative control. A comparison of PCNA+ cells and BrdU stained cells in serial sections at each time point demonstrated that the PCNA+ cells were located in the new bone formation at the osteotomy sites. No PCNA+ cells were seen in the control sections. A comparison of BrdU+ cells and HA stained cells in serial sections at the 2 and 24 hour time points demonstrated that BrdU+ cells were also located outside the new bone formation. At 24 and 48 hours post operation, BrdU+ cells first appeared scattered throughout the proximal new bone, microcolumns, and cartilage. This same pattern was observed at the 72 hour time point, except that a greater number of cells in the new columns were labeled. The results demonstrate for the first time that during DO at least a subset of the proliferating cells, juxtaposed to the newly developing osteoid columns tips, can be "tracked" over a 48 to 72 hour period, into the osteoid columns confirmaing their identification as preosteoblasts.

**Disclosures:** C.K. Lumpkin, None.

**M045**


Angiogenesis has been shown to play a key role in bone regeneration and fracture repair. We previously demonstrated that COX-2−/− mice exhibited impaired fracture healing, as evidenced by significant delay in mesenchymal differentiation and cartilage mineralization. To further determine whether delayed mineralization in COX-2−/− mice was due to the deficiency in neovascularization, we used micro-computed tomography (Micro-CT) to examine the quantity and quality of vascularization in the fracture callus of COX-2−/− mice and their wild type control mice. A mid-diaphyseal fracture was created via 3 point bending in mouse femur and subsequently stabilized by a metal pin placed in the intramedullary canal. Micro-CT vascular imaging was obtained via perfusion of a lead chromate based contrast agent (Microfil) followed by complete decalcification. Segmentation was performed using software provided by Scanco to contour the periosteal external callus and endosteal internal callus, excluding the cortex of the fractured femur. The three-dimensional reconstruction of microvascularization within callus demonstrated a dramatic reduction of neovascularization in COX-2−/− mice compared to the wild type control at day 14 post-fracture (Figure). Clusters of highly connected, closely spaced, and isotropically oriented small vessels were observed in wild type mice, but were almost absent in COX-2−/− mice. Quantitative analyses demonstrated that the volume fraction of microvasculature (Micro-CT) was reduced by 3 times in COX-2−/− mice compared to wild type mice (p<0.05, n=3). Accordingly, vessel separation was increased by 1.3 fold and the average thickness of the vessels was decreased by 26% in the lankbone (p<0.05). These data support the idea that the reduced neovascularization is responsible for the delayed bone formation and impaired healing in COX-2−/− mice. Together, our data suggests that deficiencies in angiogenesis may play a key role in delayed fracture healing in COX-2−/− mice.

**Disclosures:** X. Zhang, None.

**M044**


Microdamage (mdx) accumulation is believed to be important in the etiology of stress and fragility fractures. But detecting in vivo bone mdx is difficult. Confocal microscopy appears to be gaining popularity as the "gold standard" for detecting mdx because it is considered to be more sensitive than light microscopy. We tested this hypothesis using one raccoon each from 11 wild deer, 11 wild elk, 11 domesticated sheep, and 11 non-racing horses. In addition to examining entire bone sections, we also examined prefrontal and predominant media/lateral "shear" regions and caudal "tension" regions to see if these relatively more deleterious loading environments were associated with increased in vivo mdx when compared to cranial "compression" regions. 14mm thick mid-diaphyseal segments were built stained in 1% basic fuchsin [Buehler & Stafford 1990 Clin. Orthop.]. Two investigators, blinded to the hypotheses, rigorously examined three 100 micron-thick slices/ bone at 10-40X using transmitted light and confocal microscopy for various forms of in vivo fuchsin stained mdx (linear microcracks, diffuse mdx, wispy mdx, and osteonel debonding). Confocal microscopy was conducted using a Nikon PCM-2000 with a green HeNe LASER (543nm wavelength excitation; 555 LP filter). Evaluation is light microscopy revealed 15 linear microcracks in 132 sections; nine in deer (4 bones), two in elk (1 bone), and four in horses (3 bones). In addition to confirming the presence of the 15 linear microcracks with light microscopy in the deer, elk, and horses, evaluation using confocal revealed 83 additional mdx entities (debonding, wispy, and diffuse), none of which could be detected with light microscopy. Frequencies of additional mdx entities included: deer (n=45; all bones), elk (n=24; all bones), and horses (n=14; 9 bones). No mdx was detected in sheep calvicfem using light or confocal microscopy. These results confirm the higher sensitivity of confocal microscopy. Higher levels of physical activity may explain why the wild animals exhibited significantly more mdx. Approximately 60% of mdx was concentrated in the caudal cortices where tensile strains are prevalent/predominant, possibly accounting for the high remodeling activity in this region. Confocal microscopy may help determine if bones subject to habitual bending incur similar regional variations in mdx prevalence and/or if these regional variations are minimized by regional adaptations (e.g., osteon densities, collagen orientation) that serve to "toughen" the bone. In turn, confocal microscopy may help determine how stress distributions might increase fracture propensity in some bones, especially with aging (e.g., proximal human femur).

**Disclosures:** W.E. Anderson, None.

**M046**

**Automated Volumetric Imaging of In Vivo Bone Formation Labels.** G.J. Karakiz*, M. Singh*, W. Yao*, N. E. Lane*, T.M. Kesvey*, 1UC, San Francisco, CA, USA, 2UC, Berkeley, CA, USA, 3Stanford, Stanford, CA, USA, 4UC Davis, Sacramento, CA, USA.

In vivo administered fluorescent labels are indispensable in the study of bone metabolism. Standard histomorphometry - particularly in mineralized tissues - is technically challenging and labor intensive. Moreover, histomorphometric measures based on this sections also rely only 2D measures of the 3D structures of interest. With renewed interest in the role of bone quality in osteoporosis, the distinction between 2D and 3D measures of histological structures such as remodeling spaces and microdamage may be important. The goal of this work was to apply a novel technique for visualizing 3D distribution of fluorochrome labeled components within large biological specimens. This method is based on computer controlled milling technique and combines an arrayed imaging technique with fluorochrome capabilities, thus enabling 3D fluorescence imaging of bone specimens of any size at high resolution (on the order of a few microns). Biological specimens containing fluorescent labels are imaged in an opaque resin and mounted onto the system for serial milling and imaging. After each milling pass, an imaging station excites the exposed fluorochromes and captures the emitted fluorescence through a series of filters. As an example, an in vivo calcified labeled femur from an six month old female Sprague-Dawley rat was harvested and imaged. The proximal femur was fixed in 10% NBF, dehydrated and embedded in opaque MMA. Auroluciferase was exploited to capture bone morphometry using an UV excitation filter and a DAPI emission filter. CalfEosin fluorescence was imaged using a FITC filter set. Two 2x3 image stacks (one per filter set) were captured for each expanded surface. A 5 mm length was imaged using 680 slices at an in-plane resolution of 3 microns/pixel and an out-of-plane resolution of 8 microns. Two superimposed volumetric data sets were rendered for visualization - one representing bone morphology and the other representing bone formation zones. Spatial distribution of bone forming zones and their relation to local bone architecture were clearly distinguished and substructures such as an entire ostoon (length 1.36 mm; mean diameter 129 microns) were isolated. Imaging time...