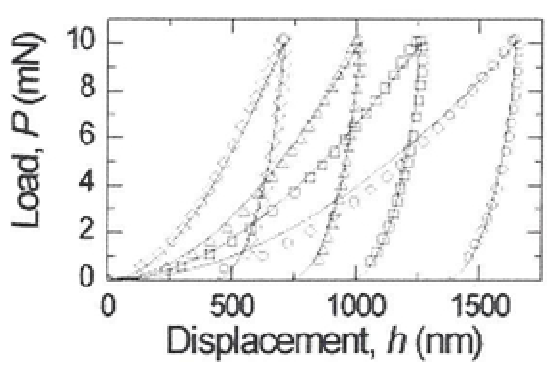
molecular mechanisms of osteopontin expression in response to mechanical stress loading or sex hormone depletion, we produce transgenic mice lines carrying GFP (green fluorescence protein) under the control of various lengths of osteopontin promoter. These transgenic mice and osteopontin knockout mice were treated as follows. 1) Orthodontic closed coil springs were bonded to maxillary first molars and incisors for the experimental tooth movement and maxillary first molars were mesially moved by continuous force (approximately 10g). 2) The mice were surgically ovariectomized or injected with leuplin, an antagonist of LH-RH receptor. Expressions of endogenous osteopontin and GFP were examined by in situ hybridization and immunohistochemistry and compared with each other. OPN mRNA-expressing cells were detected in a population of osteocytes, osteoclasts and osteoblasts on the alveolar bone surface after 72 hours of treatment in wild-type (non-transgenic) mice. The localization of GFP in Op5.5GFP mice was consistent with that of endogenous osteopontin during tooth movement or sex hormone depletion. On the contrary, the localizations of GFP-expressing cells in Op3.1GFP and Op1.5GFP were different from that of Op5.5GFP mice. Moreover, expression levels of GFP were decreased in Op3.1GFP and Op1.5GFP transgenic mice and were undetectable in Op0.9GFP transgenic mice. Taken together with the results obtained by the analysis of OPN-knockout mice, our experimental system and result may provide us with a clue for the elucidation of the molecular mechanism of bone remodeling caused by mechanical stress loading or sex hormone depletion.

Disclosures: S. Fujihara, None.

M049

Local Variations in Viscous-Elastic-Plastic Nanoindentation Responses of Healing Bone. M. L. Oyen*[1], C. Ko*[2]. [1]Biophysical Sciences, University of Minnesota, Minneapolis, MN, USA, [2]Oral Sciences, University of Minnesota, Minneapolis, MN, USA.

Information is scarce about mechanical properties of healing bone. In this study, viscoelastic and plastic properties of healing bone (adjacent to a dental implant) were assessed by nanoindentation experiments. The 4th premolar of a two-year-old Sinclair miniswine was surgically removed unilaterally (Animal Protocol #9910A2261). A smooth titanium threaded dental implant was inserted in the alveolar ridge after the extraction wound had healed for 7 months. The implant was protected from bite forces for 1 month, then the animal was sacrificed. Bone samples were harvested and embedded in polymer resin, sectioned, and polished to 0.5 μm for testing. Mechanical tests were performed in air with an MTS Nanoindenter XP using a Berkovich indenter, to a peak load of 10 mN at fixed loading and unloading rate of 0.33 mN/s. Spatial position was recorded for each test and used to determine the distance from the bone-implant interface. The load-displacement traces were analyzed using a new technique called the VEP model, which estimates viscous, elastic, and plastic coefficients for the material [J Mater Res 2003; 18(1):139-50]. Nanoindentation tests performed near the implant interface (<0.25 mm) showed substantial variations from point to point, as shown by four sample traces in the Figure (hollow symbols). It was found that the VEP indentation model could well-approximate load-displacement traces for bone, as shown by the solid lines in the Figure. Surprisingly, elastic modulus and viscosity terms were unchanged for the different locations (E = 16 GPa, time constant τ = 101 s), and all variations in the responses could be attributed to the Tabor hardness term, which measures resistance to permanent deformation. The Tabor hardness values varied from 0.28 to 5.15 GPa near the interface, and converged to 2.0 ± 0.3 GPa for tests > 2.5 mm from the interface. Nanoindentation testing revealed substantial variations in mechanical response near a bone-implant interface, which undergoes *de novo* mineralization (1-month healing). Application of a new technique for indentation analysis of viscoelastic-plastic materials showed that the viscoelastic properties remained unchanged, and that the variations could be all attributed to variations in the local Tabor hardness of the mineralized tissues.



Disclosures: M.L. Oyen, None.

M050

58. **Does Laminarity Mediate Site-Specific Differences in Collagen Fiber Orientation in Primary Bone? An Evaluation in the Turkey Ulna.** J. G. Skedros, K. J. Hunt. Dept Orthopaedics, University of Utah, Salt Lake City, UT, USA.

It has been hypothesized that preferred orientations of primary vascular canals in primary cortical bone mediate important mechanical adaptations [de Margerie 2002, J Anat]. Specifically, bones that receive habitual compression, tension, or bending stresses typically have cortices with a low laminarity index (LI) (i.e., relatively low circularly (C) oriented primary vascular canal area, and relative higher areas attributed to canals with radial (R), oblique (O), or longitudinal (L) orientations). In contrast, bones subject to habitual torsion have high LIs (i.e., relatively greater areas attributed to C-oriented primary vascular canals). Bone ultrastructure, characterized by regional variations in predominant collagen fiber orientation (CFO), may be the adaptive characteristic mediated by the degree of laminarity. We tested the hypothesis that site-specific variations in predominant CFO in the turkey ulna diaphysis strongly correlate with the degree of laminarity. Thin cross-sections (100+/-5 mm) from mid-diaphyses of 11 sub-adult and 11 adult domestic turkey ulnae were evaluated for predominant CFO and LIs using circularly polarized light images [Laminarity index (LI; based on %vascular canal area) = C/(C+R+O+L)]. Results showed that the mean LI of sub-adult bones (40 +/- 11) is significantly less than adult bones (51 +/- 10) (p<0.01). This suggests that adult bones experience relatively more prevalent torsion compared to sub-adults. Moderate-to-high positive correlations were shown between LI and predominant CFO (sub-adults: r = 0.735; adults: r = 0.866; p<0.001). These data support the hypothesis that a causal relationship may exist between these variables. Furthermore, these data indicate that primary bone can exhibit site-specific material adaptations independent of secondary osteon formation. These results corroborate the concept that the sub-adult turkey ulna is subject to relatively more prevalent bending (relatively lower mean LI) compared to the torsionally loaded adult ulna (relatively higher mean LI). If predominant CFO and LI are sufficiently sensitive and specific parameters for inferring a bone's loading history, then these characteristics may be a reliable way of discerning regional strain environments when the application of strain gauges is difficult or impossible. We speculate that 'non-traditional' parameters such as energy absorption and fatigue resistance may be the mechanical enhancement mediated by these important material characteristics. Regional heterogeneities of primary bone organization may also be an important issue for investigating bone fluid-flow dynamics.

Disclosures: J.G. Skedros, None.

M051

Collagen Status and Brittleness of Human Cortical Bone in the Elderly. T. M. Keaveny[1], G. E. Morris*[1], E. K. Wong*[1], M. Yu*[1], A. N. Sakee*[2], N. Verzijl*[2], R. A. Bank*[2]. [1]Orthopaedic Biomechanics Laboratory, Dept. of Mechanical Engineering, UC Berkeley, Berkeley, CA, USA, [2]TNO Prevention and Health, Gaubius Laboratory, Leiden, Netherlands.

It is widely believed that factors other than bone density play a role in bone strength, and ultimately in fracture risk assessment for osteoporosis. Highly brittle bone is more susceptible to fracture during impact events such as a fall. The goal of this study was to determine if collagen status affects cortical bone brittleness among the elderly since this may provide the basis for development of new strategies for bone quality assessment. Using 88 cadaver bone specimens obtained from 42 elderly cadavers (23 males age=73.6±10.7 years, 19 females age=75.7±11.4 years), we measured the correlation between the tensile energy to failure of machined specimens of human cortical bone and a variety of parameters of collagen biochemical status as measured by HPLC. Low values of energy to failure indicate that the bone is relatively brittle. Results indicated that energy to failure was positively correlated (p<0.05 in all cases) with percent collagen content (r=0.26), Hyl (r=0.25), LP (r=0.21), and HP (r=0.30), and was negatively correlated with pentosidine (r=-0.22). As expected, energy to failure was also negatively correlated with cortical bone porosity (r=-0.47). These findings indicate that the status of the collagen plays a small but significant role in brittleness of elderly human cortical bone. Specifically, elderly individuals having high levels of non enzymatic crosslinks and low levels of enzymatic crosslinks and collagen content tend to have more brittle bone, and thus may be at elevated risk of fracture in the event of a fall.

Disclosures: T.M. Keaveny, None.

Abstracts