**INTRODUCTION**

Cortical bone is a viscoelastic material, as evidenced by its creep and relaxation behavior [1]. The viscoelastic properties are correlated with the ultimate strength and toughness [2], suggesting they play a role in determination of fracture risk. However, because bone is a hierarchical composite, the viscoelastic properties may differ for at the microstructural level, with the overall behavior depending on their interactions. Understanding the microstructural basis of the viscoelasticity may provide insight into bone fragility.

Nanoindentation is an effective method for measuring mechanical properties of bone at the microstructural level [9]. Nanoindentation was used to measure the viscosity and indentation modulus in osteons of canine samples [4], which were positively correlated. However, the dependence of viscoelastic properties on age and gender within the microstructure of human cortical bone has not been studied.

The goal of this study was to investigate the dependence of the microstructural viscoelastic properties of human cortical bone. The specific aims were to 1) measure the modulus and creep time constant of human cortical bone in osteons and interstitial tissue; and 2) investigate the dependence of the properties on age, gender, and microstructural location.

**METHODS**

Twenty human cortical bone samples were studied. Samples were prepared from the femora of ten males (mean age 70 ± 12 yrs ranging from 51-87) and ten females (76 ± 11 yrs ranging from 58-89). Small cubes, approximately 5 mm thick, were cut from the mid-diaphysis using a diamond saw. The surfaces were polished with successive grades of abrasives ending with 0.25 μm alumina suspension. The bone was kept hydrated with buffered saline throughout cutting and polishing.

All of the samples were subjected to nanoindentation. Each sample was indented 20 times in ostensible tissue and 20 times in interstitial tissue using a Berkovich pyramidal indenter. The indenter was advanced at a rate of 2.0 mN/s to 10 mN, held at constant load for 10 seconds, and unloaded at 2.0 mN/s. The modulus was calculated using the Pharr-Oliver relationship.

The viscoelastic properties were found by fitting the indentation depth-time curve to a Maxwell-Voigt model given by:

\[
h(t) = \frac{\pi P_0 \alpha}{2} \left[ \frac{1}{E_1} + \frac{1}{E_2} \right] (1 - e^{-t/\tau}) + \frac{t}{\eta_1}
\]

where \(h(t)\) is the indentation depth, \(P_0\) is the peak force, \(\alpha\) is the equivalent cone semi-angle, (70.3° for a Berkovich indenter), \(E_1\) and \(E_2\) are moduli (GPa), \(\tau\) is the creep time constant (s), and \(\eta_1\) is the long term creep viscosity (GPa•s) [5]. All four parameters were determined for each indent, and averaged within samples.

The dependence of creep time constant on age, gender, tissue type, and elastic modulus were examined using ANCOVA (JMP).

**RESULTS**

The elastic modulus of the interstitial tissue, based on the Pharr-Oliver relationship was 20.15 ± 3.51 GPa, while that of the osteonal tissue was 16.50 ± 3.46 GPa (p < 0.003). The modulus did not depend on age (p > 0.8) or gender (p > 0.7) for either tissue type. Within a sample, the maximum coefficient of variation of the modulus measurements was 18.18%.

The Maxwell-Voigt model was able to capture the time dependent creep behavior (r² > 0.99, p < 0.001 for all samples; Fig. 1). The coefficient of variation of the creep time constant was 23.2%, on average.

The creep time constant was similar for ostensible and interstitial bone tissue (p > 0.1), and did not depend on age (p > 0.2). However, the creep time constant decreased with increasing modulus (p < 0.003), (Fig. 1). The adjusted mean time constant was 1.37 ± 0.18 s for females vs. 1.20 ± 0.24 s for males (p < 0.04).

**DISCUSSION**

The viscoelastic properties of bone may play a role in remodeling [6] and in strength [2]. Using nanoindentation, we found that on the scale of individual osteons, bone from females had a greater creep time constant than males regardless of age or modulus. To our knowledge, this is the first report of differing viscoelastic properties between male and female bone at either the macroscopic or microstructural level.

The strength of this study was that measurements were made from individuals over a wide age range, and in both osteons and interstitial bone. The primary limitation is the absence of mineralization measurements, which could provide insight into the results.

The results are consistent with previous nanoindentation and macroscopic studies. The interstitial tissue, which is more highly mineralized, exhibited a higher modulus [7]. The negative correlation between the modulus and creep time constant is consistent with macroscopic tests where the time constant increased with water content [8], which is in turn negatively correlated with modulus. However, the relationship between the viscoelastic parameters at the microstructural and macroscopic levels is not fully understood. The time constants found here were an order of magnitude lower than the macroscopic values [8]. Fluid flow and cement line interactions have been suggested as possible factors contributing to the macroscopic viscoelasticity of bone [1], and they may dominate the behavior at the macroscopic level.

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**REFERENCES**