Robust Method for Mechanical Testing of Rat Vertebrae to Determine Compressive Bone Properties

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Introduction: Animal models, specifically rats and mice, have been shown to provide an attractive platform to study changes due to experimental drugs, genetic knockouts, and are small enough for space missions.[1] Our research is focused on understanding the effect of radiation exposure during extended spaceflight, which will be important as the National Aeronautics and Space Administration (NASA) plans on longer manned missions in space.[2] However, mechanically testing bone specimens from mouse specimens has been challenging, due to the small specimen size for which small variations in sample preparation can appreciably alter the measured mechanical properties. Recently, we developed a protocol to semi-automate sample preparation of mouse vertebra specimens for compression testing.[3] The objective of this study was to validate the robustness of using our semi-automated sample preparation method to acquire compressive mechanical properties of rat vertebral bodies.

Materials and Methods: Rat lumbar spine specimens (n = 11) were acquired using IACUC approved protocols. Surrounding musculature was removed and the third lumbar (L3) vertebra was extracted by cutting through the adjacent intervertebral discs. Each L3 vertebra was housed in a 3D-printed fixture with a pin running through the spinal canal to ensure alignment, and the vertebral posterior process was potted in bone cement. The cranial endplates were removed by making parallel cuts at the superior and inferior vertebral bodies, to improve load distribution for uniaxial compression testing. The specimen was extracted from the cement using a Dremel saw. Two samples were monotonically compressed to failure to determine the ultimate load. The remaining samples (n = 9) were cyclically loaded between 20 N and 50% of the ultimate load to measure stiffness. To test for robustness and repeatability of measuring rat vertebrae mechanics, each sample was removed from the mechanical testing device and retested, up to five times. For this study, we used specimens that received various doses of radiation (0 – 1 Gy). While the effect of radiation was not the focus of this study, it allowed us to investigate the standard deviation for grouped specimens (inter-specimen variability, n = 2-3 per group).

Results and Discussion: Ultimate load at failure was 275 ± 55 N. The modulus measured in this study ranged from 834 – 2230 MPa, which was within the range of previous reported values for human bone specimens.[4] The average intra-specimen standard deviation was 6% (Figure 1A; maximum = 12% – 'Sample F'). The standard deviation increased slightly as values were grouped by radiation dose, resulting in an average inter-specimen standard deviation of 12% (Figure 1B; maximum = 19% – '0.5 Gy' group).

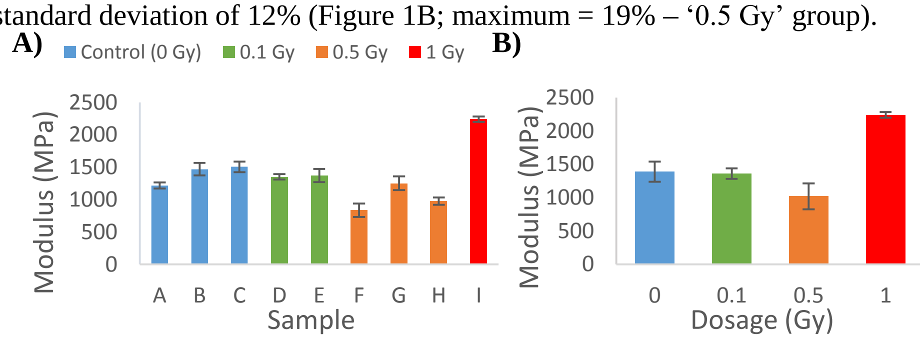

Figure 1. Compressive modulus of rat vertebrae: (A) Intra-variability for each specimen, and (B) inter-variability with respect to radiation dose. Error bars = standard deviation.

Conclusions: Together, these results indicate that the semi-automated sample preparation protocol provides measurements of mechanical properties that are highly repeatable. Our future work will investigate the effect radiation on bone mechanics; however, this method can be widely applied to understanding other mechanisms that are known to alter bone quality or quantity (e.g., drugs or genetic alterations).

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