Effect of *Ex Vivo* Ionizing Radiation on Static and Fatigue Properties of Mouse Vertebral Bodies

Shannon R. Emerzian[1], Megan M. Pendleton[1], Alfred Li[2], Jennifer W. Liu[3], Joshua S. Alwood[4], Grace D. O'Connell[1], Tony M. Keaveny[1]
[1]University of California, Berkeley, CA, [2]University of California, San Francisco, CA, [3]Washington University, St. Louis, MO, [4]NASA Ames Research Center, Moffett Field, CA,
Email: semerzian@berkeley.edu

Disclosures: The authors have nothing to disclose.

INTRODUCTION: For a variety of medical and scientific reasons, human bones can be exposed to a wide range of ionizing radiation levels. *In vivo* radiation therapy (0.05 kGy) is used in cancer treatment, and *ex vivo* irradiation (25-35 kGy) is used to sterilize bone allografts. Ionizing radiation in these applications has been shown to increase risk of fracture[1], decrease bone quality[2], [3], and degrade collagen integrity[4]. Past studies have investigated the deleterious effects of radiation on cortical or trabecular bone specimens individually, but to date no studies have examined whole bones containing both cortical and trabecular tissue. Furthermore, a clear relationship between the dose and the mechanical and biochemical response of bone's extracellular matrix has yet to be established for doses ranging from cancer therapy to allograft sterilization (0.05-35 kGy). To gain insight into these issues, we conducted an *ex vivo* radiation study to investigate non-cellular (i.e. matrix) effects of ionizing radiation dose on vertebral whole bone mechanical properties, over a range of radiation doses (0.05-35 kGy), with a focus on any radiation-induced changes in collagen. With underlying mechanisms of action in mind, we hypothesized that any induced reductions in mechanical properties would be associated with changes in collagen integrity.

METHODS: 20-week old female mice were euthanized and the lumbar spine was dissected using IACUC approved protocols. The lumbar vertebrae (L1-S1) were extracted from the spine via cuts through adjacent intervertebral discs, and the endplates, posterior processes, surrounding musculature, and soft tissues were removed (~1.5mm diameter, ~2mm height). Specimens were randomly assigned to one of five groups for *ex vivo* radiation exposure: x-ray irradiation at 0.05, 1, 17, or 35 kGy, or a 0 kGy control. Following irradiation, the vertebrae were imaged using microcomputed tomography (micro-CT) and then subjected to either monotonic compressive loading to failure or uniform cyclic compressive loading. During cyclic testing, samples were loaded in force control to a force level that corresponded to a strain of 0.46%, as determined in advance by a linearly elastic micro-CT-based finite element analysis for each specimen. Tests were stopped at imminent fracture, defined as a rapid increase in strain. The main outcome for the monotonic test was the strength (maximum force); for cyclic testing it was the fatigue life (log of the number of cycles of loading at imminent failure). A fluorometric assay was used on the S1 vertebrae to measure the number of non-enzymatic collagen crosslinks[4]. A one-way ANOVA was performed on mechanical properties and collagen crosslinks; means were compared with controls using Dunnett's method, with a Tukey-Kramer post-hoc analysis when significance was found ($p \leq 0.05$).

RESULTS: Compared to the unirradiated control group, the concentration of non-enzymatic collagen crosslinks was significantly increased for all irradiated groups ($p < 0.0001$), and being higher by at least 50% (Figure 1a). By contrast, the radiation effects on the collagen were only evident at the higher doses. For irradiation exposures of 17 kGy or more, strength decreased substantially as the radiation level was increased, but no effect was evident below 17 kGy (Figure 1b). There was no significant change in the stiffness or maximum displacement for any radiation dose ($p>0.05$). The finite element analysis-prescribed force level for cyclic loading exceeded the measured (monotonic) strength of the 17 and 35 kGy irradiated groups (mean ± SD, 20.6 ± 5.6 N; 13.2 ± 3.7 N, respectively) and therefore these groups were eliminated from the fatigue study. The fatigue life for the 0.05 and 1 kGy groups were similar to each other and were not statistically significantly different from the control group (Figure 1c).

DISCUSSION: Although an irradiation sterilization dose of 35 kGy is known to degrade the structure and mechanical properties of cortical bone allografts[4], here we demonstrate that vertebral bone tissue – at least in the mouse model - can be irreversibly damaged at doses well below this dose. This is potentially important when considering the long-term mechanical integrity of bone allografts placed in patients. Interestingly, while the strength decreased appreciably for the 17 and 35 kGy doses, the number of crosslinks was not altered for those groups. This discrepancy suggests that radiation has a greater influence on mechanical behavior than collagen crosslinking, and that the mechanism for mechanical change as a result of exposure to radiation involves more than collagen crosslinks. Further, there was no significant difference in cycles to failure between irradiation groups and the control despite the increase in collagen crosslinks. This lack of significance may be due to a small sample size (n = 3-8 per group), as a downward trend was detected. Ongoing work is focused on increasing the number of specimens. In summary, our study shows that an increase in the number of collagen crosslinks across all radiation dose groups is not the sole mechanism for mechanical degradation of bone tissue. Additional biochemical assays investigating the presence of the other mechanisms, such as cleavage of the collagen backbone[5], remain another topic of a future study.

SIGNIFICANCE: These findings demonstrate that exposure to *ex vivo* radiation decreases the mechanical integrity of the vertebral body and alters non-cellular matrix properties. Doses below those used to sterilize clinical allografts reduced strength and degraded the collagen in mouse vertebral bodies, suggesting that currently used doses for sterilization may compromise bone allografts used clinically.

REFERENCES: [1] Baxter, N et al., JAMA, 2005; [2] Wernle, J et al., J Biomech, 2010; [3] Barth, H et al., Bone, 2010; [4] Barth, H et al., Biomaterials 2011; [5] Burton, B et al., Bone, 2014

ACKNOWLEDGEMENTS: This study was supported by the UC Berkeley Chancellor's Fellowship (SRE), a NASA Space Biology PECASE (JSA), and NASA Science & Technology Research Fellowship NNX14AM56H (MMP).

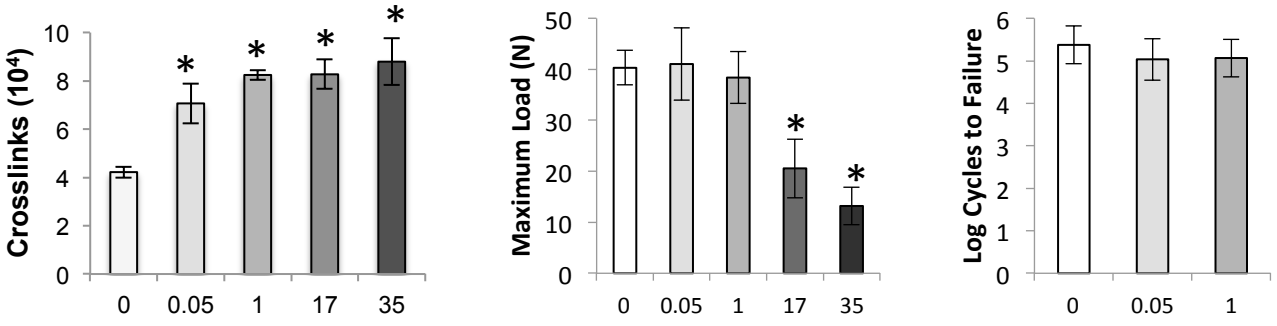

Figure 1: (a) Total number of fluorescent (non-enzymatic) crosslinks; (b) Maximum load at failure for monotonic testing; (c) Log cycles to failure for fatigue testing; [x-axis is radiation dose in kGy; error bars represent standard deviation; * = p<0.001 vs. 0 kGy control for all]