Ionizing radiation from *ex vivo* sterilization diminishes collagen integrity and vertebral body mechanics

Shannon R. Emerzian[1], Megan M. Pendleton[1], Alfred Li[2], Jennifer W. Liu[3], Simon Y. Tang[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

Background

- Clinical exposure to ionizing radiation could put cancer radiotherapy[1] or bone allograft[2] patients at an increased risk of fracture.
- In these applications, ionizing radiation levels can range from accumulative 50 Gy for radiotherapy cancer treatment, to acute 35,000 Gy for allograft sterilization.
- Ionizing radiation has been shown to decrease bone quality through reduced strength and post-yield properties[3,4] and degrade collagen integrity through either increased crosslinks (advanced glycation end products, AGEs)[4] or fragmentation[5].
- It is unclear which collagen structural change accounts for reduced strength.
- The dose-dependent effect of ionizing radiation on mechanical and biochemical properties of whole bones are not well understood, particularly for *ex vivo* doses ranging from 50 to 35,000 Gy.

Objective

- Thus, the goal of this study was to investigate the dose-dependent, non-cellular effect of ionizing radiation on collagen network structure and ultimate strength of whole murine vertebral bodies.

Methods

Experimental Design

- Lumbar vertebrae (L3, L4, S1) from 20-week old female C57BL/6 mice
- Five dose groups, n=9/group

I. Sample Preparation

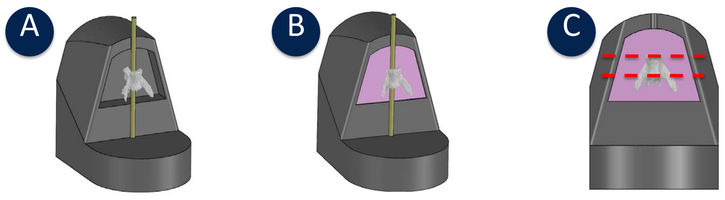

Fig. 1: Sample preparation steps for L4 murine vertebrae prior to mechanical characterization

II. Ex Vivo Irradiation (IRR)

Specimens were randomly assigned to one of five *ex vivo* x-ray irradiation dose groups: 0 (control), 50, 1,000, 17,000, or 35,000 Gy

III. Collagen Characterization

- **Crosslinks (AGEs)**:
  - Total fluorescence[4] (S1)
  - Outcome: Relative amount of AGEs (ng quinine/mg collagen)
- **Fragmentation**:
  - Automated electrophoresis (L3)
  - Outcome: Collagen molecular weight (MW) distribution via gel and electropherogram (MW in kDa; amount of protein at given MW in fluorescence unit, FU)

IV. Mechanical Characterization

- Monotonic compression to failure (L4)
- Outcome: vertebral strength (maximum force on force-deformation curve, N)

Results

- Collagen crosslinks (AGEs) increased significantly for all irradiated groups ($p<0.0001$), but these groups were not significantly different from one another ($p>0.05$; **Fig. 2A**).
- In contrast, the radiation effects on collagen chain MW distribution and monotonic strength were only evident for the doses of 17,000 and 35,000 Gy. At those doses, the amount of nominal size collagen chains (~150kDa) decreased, indicating a rise in collagen fragmentation (**Fig. 2C,D**), and the monotonic strength decreased by 50–67% ($p<0.0001$, **Fig. 2B**).

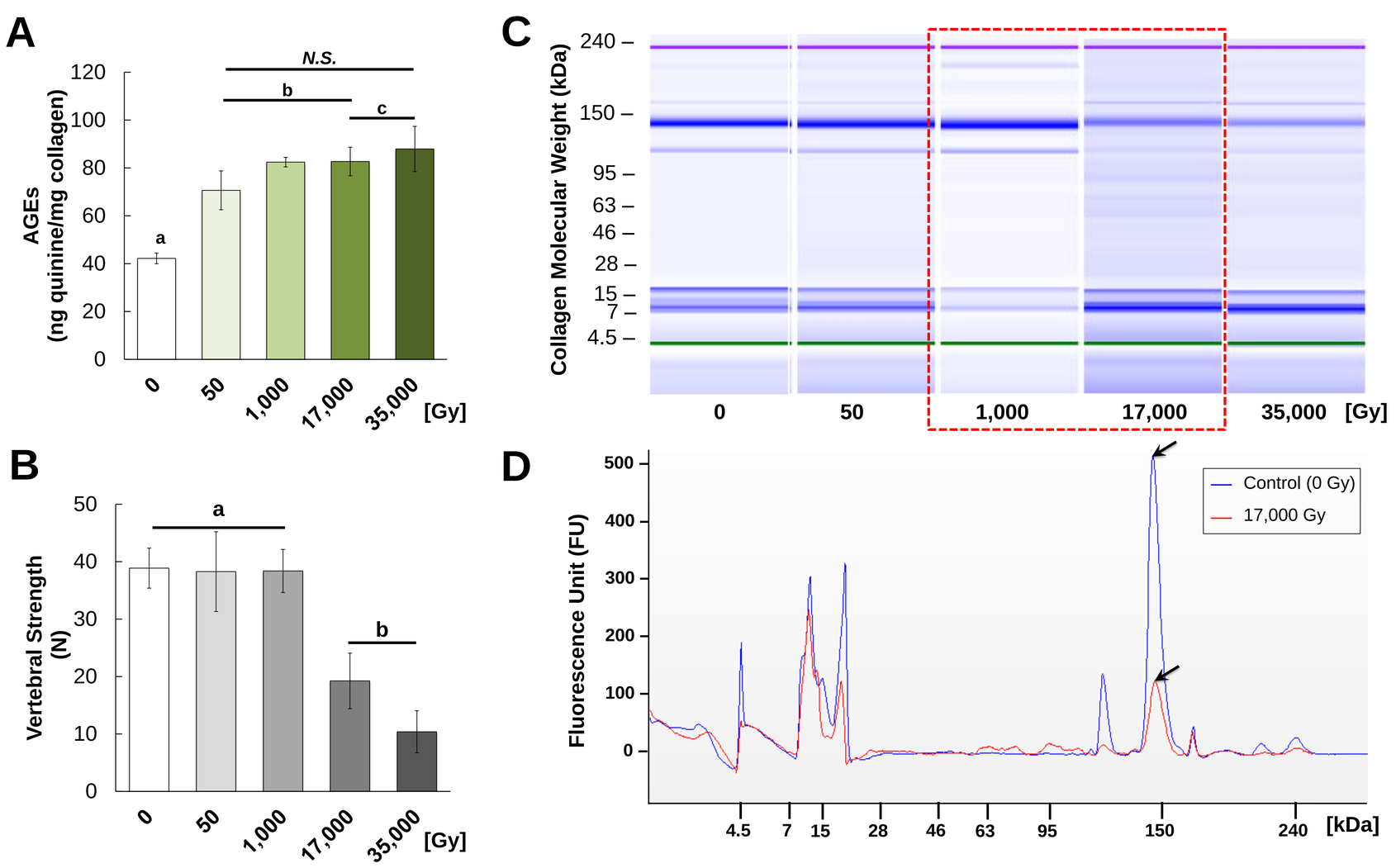

Fig. 2: (A) Crosslinks: Relative amount of AGEs versus radiation dose. **(B) Strength:** Vertebral strength versus radiation dose. **(C) Fragmentation (gel):** Exemplary bioanalyzer gel of collagen from demineralized, pepsin-digested murine bone; collagen MW versus dose. Collagen ≥17,000 Gy had a less dense band at ~150 kDa and smearing at lower MWs, indicative of fragmentation. **(D) Fragmentation (electropherogram):** Amount of collagen (FU) versus MW for control and 17,000 Gy. Error bars = 95% confidence intervals. ANOVA with Tukey-Kramer post-hoc, $p<.05$. Groups with the same lower case letters are not significantly different from one another.

Discussion

- Irreversible mechanical degradation and collagen fragmentation are observed beginning at an *ex vivo* dose of 17,000 Gy, which is two-fold lower than standard allograft sterilization (~35,000 Gy).
- Previous work suggested either increased collagen crosslinking[2] or fragmentation[3] to be the underlying cause of mechanical degradation.
- Here, we demonstrate increases in collagen fragmentation coincided with decreased strength. In contrast, the increase in crosslinks occurs for all irradiated specimens, and does not independently coincide with mechanical degradation (**Fig. 3**).

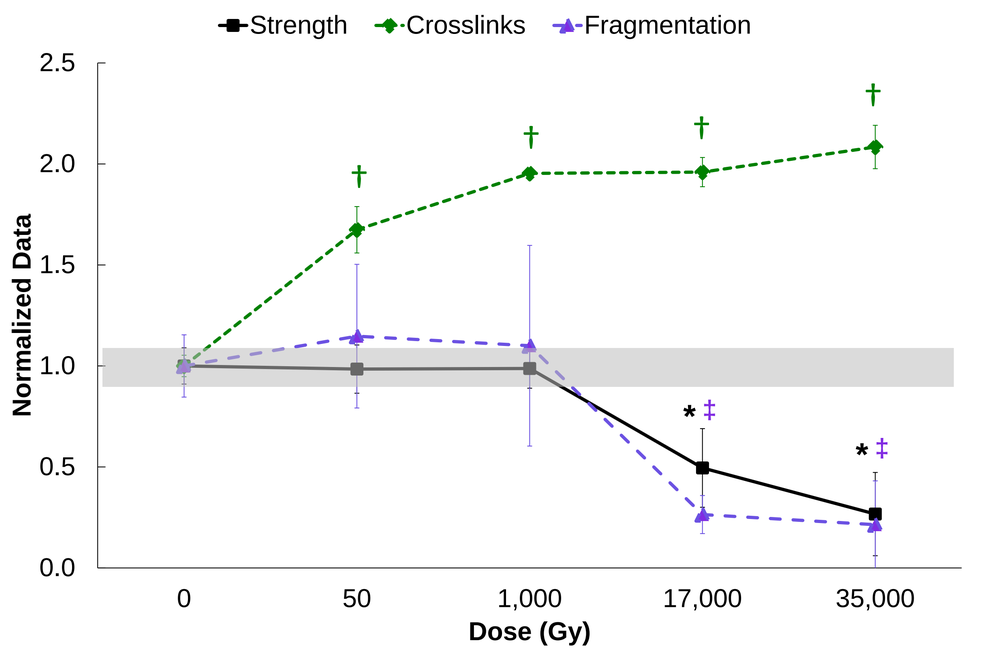

Fig. 3: Trend of normalized vertebral ultimate strength (control: 38.9 ± 3.5 N), relative amount of fluorescent crosslinks (AGEs; control: 42.2 ± 2.2 ng quinine/mg collagen), and nominal length collagen chains (~150 kDa) unbroken by radiation (FU; control: 460.1 ± 70.9 FU), versus radiation dose. Data are shown as least-square means normalized respective 0 Gy control. Error bars = 95% confidence intervals. ANOVA with Dunnett's post-hoc, $p<.05$. * represents $p<.0001$ for vertebral ultimate strength; † represents $p<.0001$ for AGEs; ‡ represents $p=.0009$ for FU.

Conclusion

- These findings raise questions regarding the mechanical integrity of bone allografts sterilized above 17,000 Gy due to fragmentation of collagen.
- In order to maintain strength following sterilization, allografts should be sterilized at a dose below 17,000 Gy whenever possible[6], or safeguarded with a radioprotectant that counters the effects of collagen fragmentation[7].

References: [1] Baxter+ *JAMA*, 2005; [2] Lietman+ *Clin. Orthop. Releat. Res.*, 2000; [3] Wernle+ *J.Biomech*, 2010; [4] Barth+ *Biomaterials*, 2011; [5] Burton+ *Bone*, 2014; [6] Nguyen+ *J.Arthroplasty*, 2011; [7] Willett+ *J. Mech. Behav. Biomed. Mater.* 2015

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