The Effects of 4-He Radiation on the Material Properties of Bone

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Introduction

NASA has a long-term goal to go to Mars. The long duration spaceflight has many risks to astronaut health, including the risk of bone loss due to the absence of gravity and exposure to space radiation [1]. There have been studies on the microstructures and structural properties on the effects of space radiation on bone, but not on the material properties. This project looks at the changes in the viscoelastic material properties of the effects of helium radiation on bone in rats using spherical micro-indentation testing.

Methods

Study Design

All animal work was performed under IACUC approved protocols of Johns Hopkins University as well as Brookhaven Laboratory. No live animal work was performed at The College of New Jersey. In this study, Long Evans Rats (n=78) were exposed to whole body helium radiation of varying doses (0, 5, and 25 cGy) at Brookhaven National Laboratory. Animals of each dose were euthanized at various timepoints after exposure (7, 30, 90, and 180 days) (Table 1) and hind limbs were removed and stored in 70% ethanol prior to testing.

	7 days	30 days	90 days	180 days
0 cGy	4	4	5	8
5 cGy	5	4	8	12
25 cGy	5	5	8	10

Table 1: *The number of animals per treatment group*

Embedding and Polishing

The samples were dried and then embedded in epoxy resin. The resulting disks were cut with a diamond saw and polished with a series of increasingly fine grit papers, followed by a polishing cloth with 0.04 µm silica suspension (Figure 1).

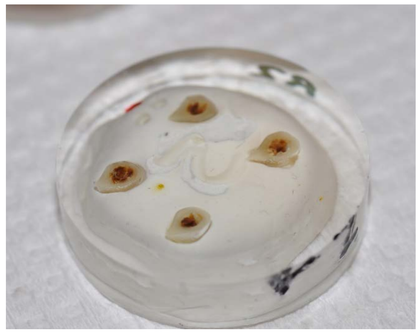

Figure 1*: The polished and embedded samples in an epoxy disk.*

Indentation Testing

A spherical micro-indenter outfitted with a DinoLite microscope was used. At each anatomical location (Figure 2), where areas to be indented were picked based on the local porosity. A 150 µm radius ruby tip was used. The target depth was 15 µm with a 30 second hold. Force-displacement data was measured with a 10 N load cell (0.1 N resolution) at a sampling rate of 200 Hz.

Indentation Testing (continued)

Samples were rehydrated prior to testing, which would aim between the pores. The resulting force-displacement data was analyzed by fitting the force relaxation response to a 3-parameter viscoelastic Maxwell solid to obtain the instantaneous and relaxed shear moduli [2,3].

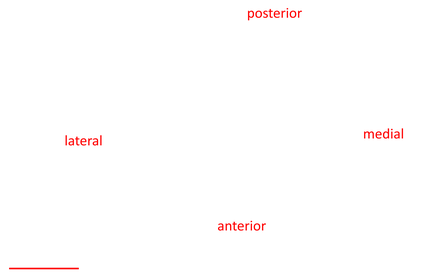

Figure 2*: A micro-indentation test on the lateral area of the specimen*

Statistical Analysis

The resulting shear moduli were statistically analyzed using a standard least squares model. The dosage of radiation, the time after exposure, and the location of the indent were all considered fixed effects, whereas each bone specimen was considered a random effect. Effect tests and post-hoc Tukey tests were performed for each interaction.

Results

The variables of dose, time after exposure, and the interaction between dose and time were all considered to be significant effects. Within dosage, the post-hoc Tukey test showed that the 25cGy treatment was statistically different for both the instantaneous and relaxed shear moduli from both the 0 and 5cGy treatments (Figure 3, top). For the timepoint variable, the post-hoc Tukey test identified that the 90 day timepoint was different from 7, 30, and 180 days after exposure for both the relaxed and instantaneous shear moduli (Figure 3, middle). On the graph, there was a distinct dip at 90 days. The post-hoc Tukey test for the interaction between time and dose showed that 25 cGy and 90 days was statistically different from every other group (Figure 3, bottom).

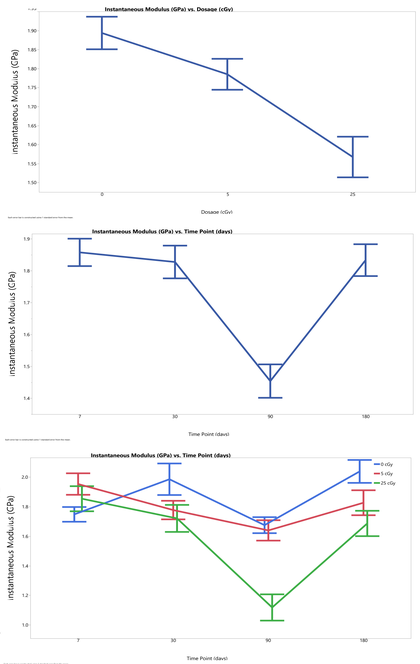

Figure 4:
Top: The shear instantaneous modulus due to different dosages of exposure with.
Middle: The shear instantaneous modulus at times after exposure
Bottom: The shear instantaneous modulus over time separated by dose.
Note: All bars are standard error bars.

Discussion

Radiation dose caused changes in the shear moduli of bone, and the effects varied with time after exposure based on healing. It is known that radiation damages osteoblasts and their precursors, which suppresses bone formation, changing bone metabolism (cite source 2 frm paper). Higher doses cause more damage, which contributes to the decline of shear moduli. It is also known that a osteoblast precursor, mesenchymal stem cells (MSCs), take between 1-3 weeks to differentiate into osteoblasts [4]. At minimum, it takes 3 weeks to start repairing bone after exposure, which means about a month has passed with minimal bone formation. The response at the 90 day timepoint fits within the proposed mechanisms of short term cell death followed by a decline, and then recovery when bone turnover is fixed by 180 days.

Conclusions

Radiation dose and the time after exposure to helium-4 radiation (25cGy) were found to cause changes in bone material stiffness when assessed with spherical micro-indentation. There was a decline at 90-days post exposure, with a partial recovery by 180-days. This decline in bone stiffness, without any compensating increase in bone volume, would increase fracture risk. Future work could further investigate the mechanisms that might cause this biological response, to identify potential therapeutic countermeasures against these degenerative changes from radiation exposure.

Acknowledgements

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References

[1] S.A.J. Lloyd, Adv Space Res, 2008, 12, 1889-1897. [2] J.M. Mattice, A.G. Lau, M.L. Oyen, R.W. Kent, Spherical indentation load-relaxation of soft biological tissues, J. Mater. Res. 21 (2006) 2003–2010. [3] A. Lau, M.L. Oyen, R.W. Kent, D. Murakami, T. Torigaki, Indentation stiffness of aging human costal cartilage, Acta Biomater. 4 (2008) 97–103. [4] M.F. Pittenger, D.E. Discher, B.M. Péault, D.G. Phinney, J.M. Hare, A.I. Caplan, Mesenchymal stem cell perspective: cell biology to clinical progress, Npj Regen. Med. 4 (2019). https://doi.org/10.1038/s41536-019-0083-6.