Effect of Radiation on Articular Cartilage Mechanical Properties

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Statement of Purpose: For astronauts, one of the harmful outcomes of spaceflight is bone loss. This is in part due to the change in mechanical stimuli caused by the skeletal unloading that occurs in microgravity. In addition, unloading effects cartilage, a tissue that has a very limited capacity for healing because it is avascular and has a low cell density. Therefore, damage that occurs in space flight could have long-term effects on astronauts' joint health. In addition to microgravity, astronauts are also exposed to complex radiation while in space. Recent studies suggest that radiation may compound microgravity's effects on bone [1, 2]. However, radiation effects on cartilage are poorly understood. Additionally, many cancer patients undergo radiotherapy as a standard treatment. Radiotherapy also puts patients at risk of damaging or altering the mechanical properties of their articular cartilage. Direct ex vivo mechanical testing of cartilage in animals (usually mice) has been difficult in the past due to the small size of the samples. However, direct ex vivo mechanical testing of soft, viscoelastic tissues from small animal models is now possible due to recent advances in techniques such as atomic force microscopy (AFM) [3]. In this study, we characterized the effects of radiation on murine articular cartilage mechanics and properties.

Methods: To maintain consistency with other studies, C57BL/6 female mice were used as the subjects in this study (N = 6 in each group). The experimental group was given a 2 gray, whole body dose of 125 peak kilovoltage photons (X-rays). The mice were then humanely euthanized 7 days after being irradiated. The articular cartilage on the distal end of the femur was the site used for mechanical testing and histological analysis. In order to replicate the mechanical properties seen while living, each femur was mechanically tested within 14 days of euthanization. Each femur was stored in Hank's balanced salt solution. The articular cartilage was mechanically tested using AFM contact mode in Hank's balanced salt solution. A 2.5 μm diameter spherical tip with a spring constant of 0.12 N/m was used as the indenter. Force versus indentation depth curves were obtained by indenting the cartilage to about 1 micron and retracting at varying speeds from 1μm/sec to 14 μm/sec. The Hertz model for a spherical indenter was used to calculate the elastic modulus of cartilage [4]. The approach data of the force versus indentation depth curve was used to calculate the elastic moduli as a function of indentation depth. The modulus values for the first 250 nm of indentation were averaged to get an estimated modulus for each sample. Cartilage is a viscoelastic tissue while the Hertz model represents only simple linear elastic response. However, the model is useful as a first approximation of cartilage nanoindentation response [5]. Samples were also fixed for histological analysis and stained with Safranin O, which allows for qualitative assessment of proteoglycan content.

Results: As expected, tissue modulus estimated from the Hertz model increased with increasing indentation speed (from 1 to 14 μm/s). In addition, the modulus did increase with indentation depth. The estimated elastic modulus of the irradiated cartilage was significantly lower than the elastic moduli for the non-irradiated samples as seen in Figure 1. The average elastic modulus for the control samples was 488.17±118.55 kPa (85% confidence interval), while the average for the irradiated samples was 3.82±1.66 kPa.

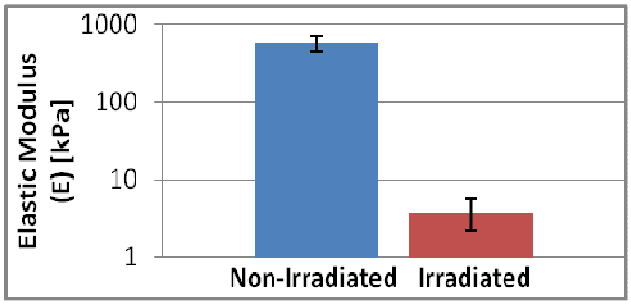
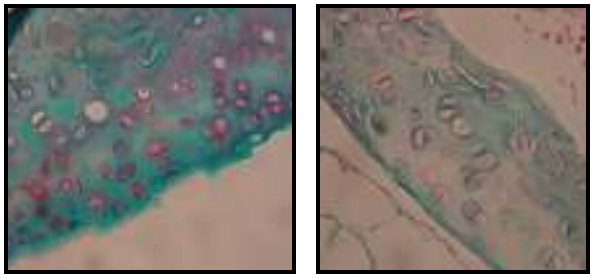


Figure 1. Elastic moduli of irradiated and non-irradiated cartilage at 14 μm/s indentation speed with an 85% confidence interval.



Figures 2 & 3. Proteoglycans (stained pink) in articular cartilage at a total magnification of 400x. Figure 2 (on left) is non-irradiated cartilage. Figure 3 (on right) is irradiated cartilage.

Cell number and gross morphology of the tissue was similar for both the groups. However, staining with Safranin O showed a decrease in proteoglycan staining in samples that were irradiated (Fig. 2&3).

Conclusions: Since the average elastic modulus of the irradiated cartilage was around two orders of magnitude smaller than the non-irradiated cartilage, we can conclude that irradiated articular cartilage is much less stiff than non-irradiated cartilage. Therefore, radiation could have detrimental effects on the function of articular cartilage. A difference in elastic moduli this large provides compelling evidence to the damage that radiation can cause to cartilage, whether it is exposure in space for astronauts or radiation treatment for cancer patients.

References: 1. Hamilton et al. J Appl Physiol. 2006; 101: 789-793. 2. Bandstra et al. Radiat Res. 2008; 169: 607. 3. Cao et al. J Biomech Eng. 2006; 128: 766-771. 4. Hemmer et al. J Eng. Proc IMechE Part H: 2008; 22: 761-772. 5. Darling et al. Osteoarthr Cartil. 2006; 14: 571-579.

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