Effect of X-Ray Irradiation on Porcine and Murine Cartilage Modulus

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Statement of Purpose: Radiation exposure as a part of standard multimodality cancer treatment has been shown to put patients at risk of tissue damage to the normal tissue surrounding the tumor being treated and possible long-term consequences as a result of this damage [1]. Astronauts are also exposed to solar radiation while in space, which has been shown to have detrimental effects on bone structure and function [2]. While the effect of radiation exposure on bone has been quantified, the effects of radiation on articular cartilage biomechanics are not well understood. Because of advances in techniques such as atomic force microscopy (AFM), the direct ex vivo testing of soft, viscoelastic tissue of small animal models, such as mice, is now possible [3]. Our studies have focused on characterizing cartilage mechanics and properties using AFM and microindention. Our prior nanoindentation study of murine samples indicated significant differences in tissue modulus after irradiation [4]. The aim of our current study is to determine if our previously observed decreases murine cartilage modulus due to radiation stem from surface changes in the tissue which is measureable by AFM testing, or if the radiation penetrates throughout the tissue as a bulk effect. AFM mechanical testing is limited to only 1-2 μm, while we found the average thickness of murine articular cartilage was 69±20 μm (Figure 1).

Methods: In the current study, porcine articular cartilage from the distal end of the femur was explanted. The experimental group was irradiated with a 2 gray dose of 125 peak kilovoltage photons (X-rays). The experimental and control samples were then tissue cultured in standard conditions using a cartilage media [5]. The articular cartilage was mechanically tested using AFM in cartilage media. For nanoindentation, a 0.3 μm radius spherical tip with a spring constant of 0.17 N/m was used as the indenter. Force versus indentation depth curves were obtained by indenting the cartilage 1-2 μm and retracting at 1 μm/s. The Hertz model for a spherical indenter was fit to the initial portion of the indentation curve to calculate the elastic modulus of cartilage [6]. 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 [7].

Results: The estimated elastic modulus of the irradiated cartilage was significantly lower than the elastic moduli for the non-irradiated samples. For the murine study, the average elastic modulus 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. For the porcine explant study, the average elastic modulus the control samples was 45.78±8.95 kPa, while the average for the irradiated samples was 20.09±8.63 kPa (Figure 3).

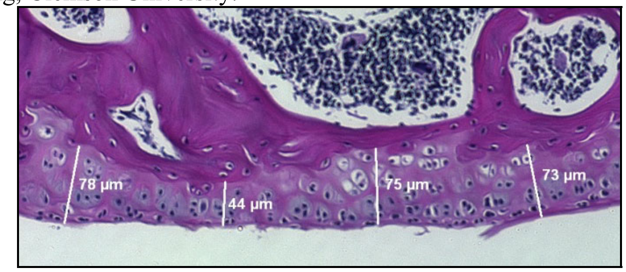
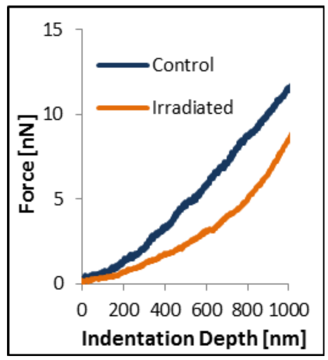


Figure 1. Cross section of the distal end of a murine femur stained with hematoxylin and eosin.



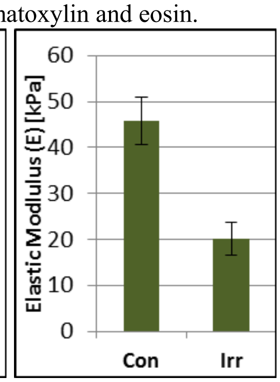


Figure 2. Sample AFM force versus indentation depth curves for control and irradiated porcine samples.
Figure 3. Average elastic modulus for control and irradiated porcine samples with an 85% CI.

Conclusions: We can conclude that irradiated articular cartilage is less stiff than non-irradiated cartilage due to the significantly lower modulus values for irradiated samples. Additionally, the tissue modulus estimated from the control samples was much lower than the values we estimated for the murine study. This can be attributed to using a lower indentation speed (1 instead of 14 μm/s), and using a smaller indenter (0.3 instead of 2.5 μm radius) [8]. However, the irradiated cartilage from the porcine study was stiffer than the murine study. This could be due to the difference in environment after irradiation (tissue culture instead of Hank's balanced salt solution). Our current work focuses on performing larger-scale mechanical tests using a UMT-2 CETR Scratch System to determine if the radiation is merely creating a surface effect on this tissue. The differences seen through both studies provide compelling evidence to the detrimental effects that radiation can cause to cartilage, whether it be exposure during spaceflight or cancer radiotherapy.

References: **1.** Levin WP et al. Br. J. Cancer. 2005; 93: 849-854. **2.** Bandstra et al. Radiat Res. 2008; 169: 607. **3.** Cao et al. J Biomech Eng. 2006; 128: 766-771. **4.** Lindburg et al. Trans. Soc. Biomat. 2010. **5.** Jin et al. Arch Biochem. Biophys. 2001; 395(1): 41-48. **6.** Hemmer et al. J Eng. Proc IMechE Part H. 2008; 22: 761-72. **7.** Darling et al. Osteoarthr Cartil. 2006; 14: 571-79. **8.** Stolz et al. Biophys. J. 2004; 86(5): 3269-83.

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