**Effects of Sterilization Techniques on the Biomechanical Properties of Rat Femora**

**INTRODUCTION:**
Osteosarcoma is the most common form of malignant bone tumor seen in children. The survival rate for patients with bone sarcomas has improved from less than 20% to approximately 80% with modern protocols of multidisciplinary treatment. Because of recent improvements in therapies, limb salvage surgery is now primarily performed to improve quality of life. While various methods are available for limb reconstruction, implantation of prostheses and bone grafting procedures are the most common. Due to improvements in prostheses designed for use in osteosarcoma patients, this has become the preferred method of limb reconstruction. However, the use of allograft for the reconstruction of large bone defects resulting from tumor resection is often a necessary adjunct to implantation of prostheses. The widespread use of allograft is unfortunately hampered by concerns over immunologic reaction, transmission of diseases, and religious dissatisfaction. Moreover, in some countries it is difficult to obtain allografts for social reasons. For these reasons, surgeons have been forced to rely instead upon autograft. In order to prevent cancer recurrence from the autograft material, many methods have been developed for sterilizing the resected bone for reconstruction, including irradiation, autoclaving, pasteurization, freezing, and heating. Although these sterilization methods have shown some clinical utility for performing reconstructions, complications such as infection, pathologic fracture, and nonunion remain too common. To date, no systematic study has comparatively evaluated the efficacy of these different sterilization strategies in order to ascertain their relative strengths and weaknesses. The purpose of this study was to compare the effects of irradiation and freezing on the biomechanical properties of rat femora.

**METHODS:**

**Animals** – These studies utilized twelve 3-month-old male Sprague Dawley rats (Harlan, Indianapolis, IN). Following euthanasia, both femora were dissected free of soft tissue and removed for testing. These procedures were approved by the University of Tennessee IACUC and were in compliance with all local and federal guidelines regarding the use of animals for research.

**Sterilization** – The harvested femora were randomized to one of the three following treatment groups (N=8) by body weight: 1) Control; 2) Irradiation; 3) Freezing. Control samples were immediately used for biomechanical testing. Irradiation samples were subjected to 25 Gy of gamma irradiation using a D3300 Gulmay orthovoltage treatment system (Gulmay, Buford GA) immediately prior to testing. Freezing samples were immersed in liquid nitrogen for 20 min and then warmed to room temperature in saline prior to testing.

**Biomechanical Testing** – In order to ascertain the biomechanical properties of the femora, 3-point bending tests to failure were performed. The femora were centered in a custom designed jig with the anterior side up and a monotonic ramp to failure was applied at a crosshead speed of 0.1 mm/min using an ElectroForce 3200 (Bose, Eden Prairie, MN). Force versus displacement curves were plotted in Excel and Failure Force (N) and Stiffness (N/mm) were calculated using a set of custom written macros.

**Statistical Analysis** – Significant differences between groups were assessed with t-tests. All tests were carried out using Excel (Microsoft, Redmond, WA) and p-values less than 0.05 were considered significant.

**RESULTS:**
The results of this study demonstrated that gamma irradiation significantly compromised the biomechanical integrity of rat femora (see Figures 1 & 2). Specifically, gamma irradiation significantly decreased Failure Force by 12% and Stiffness by 18%. In addition, Failure Force for the irradiated bones was significantly reduced compared to the frozen ones. In contrast, freezing had no significant effects on either Failure Force or Stiffness.

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