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Free radical scavenging alleviates the biomechanical impairment of gamma radiation sterilized bone tissue ☆

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Abstract

Terminal sterilization of bone allografts by gamma radiation is often essential prior to their clinical use to minimize the risk of infection and disease transmission. While gamma radiation has efficacy superior to other sterilization methods it also impairs the material properties of bone allografts, which may result in premature clinical failure of the allograft. The mechanisms by which gamma radiation sterilization damages bone tissue are not well known although there is evidence that the damage is induced via free radical attack on the collagen. In the light of the existing literature, it was hypothesized that gamma radiation induced biochemical damage to bone's collagen that can be reduced by scavenging for the free radicals generated during the ionizing radiation. It was also hypothesized that this lessening of the extent of biochemical degradation of collagen will be accompanied by alleviation in the extent of biomechanical impairment secondary to gamma radiation sterilization. Standardized tensile test specimens machined from human femoral cortical bone and specimens were assigned to four treatment groups: control, scavenger treated-control, irradiated and scavenger treated-irradiated. Thiourea was selected as the free radical scavenger and it was applied in aqueous form at the concentration of 1.5 M. Monotonic and cyclic mechanical tests were conducted to evaluate the mechanical performance of the treatment groups and the biochemical integrity of collagen molecules were assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The native mechanical properties of bone tissue did not change by thiourea treatment only. The effect of thiourea treatment on mechanical properties of irradiated specimens were such that the post-yield energy, the fracture energy and the fatigue life of thiourea treated-irradiated treatment group were 1.9-fold, 3.3-fold and 4.7-fold greater than those of the irradiated treatment group, respectively. However, the mechanical function of thiourea treated and irradiated specimens was not to the level of unirradiated controls. The damage occurred through the cleavage of the collagen backbone as revealed by SDS PAGE analysis. Irradiated specimens did not exhibit a noteworthy amount of intact α-chains whereas those irradiated in the presence of thiourea demonstrated intact α-chains. Results demonstrated that free radical damage is an important pathway of damage, caused by cleaving the collagen backbone. Blocking the activity of free radicals using the scavenger thiourea reduces the extent of damage to collagen, helping to maintain the mechanical strength of sterilized tissue. Therefore, free radical scavenger thiourea has the potential to improve the functional life-time of the allograft component following transplantation.

Keywords: Bone graft; Gamma radiation; Sterilization; Cortical bone; Collagen; SEM; Free radicals; Free radical scavengers; Thiourea; Biomechanical

Introduction

Each year, an estimated 450,000 allografts are transplanted in the US for repair of fractures and damage

☆ The process described herein which describes reducing the biomechanical and biochemical impairment of gamma radiation sterilized graft components is patent pending.

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caused by illness and injury, replacing bone lost in tumor removal, and reconstruction of skeletal defects. Allograft popularity is related both to its biocompatibility and to its suitability for anatomical matching to repair defects. Regardless of their expediency, risk of infection and disease transmission through allografts is a concern and terminal sterilization is often essential [26]. Gamma radiation has been widely used for sterilization of bone allografts due to its efficacy against viral and bacterial disease transmission [5,6,8]. However, gamma radiation sterilization impairs the material properties of bone [1,2,5,12,18,39] which is a major clinical concern since bone grafts are used in load bearing applications [13].

In assessing the mechanical properties of bone irradiated with gamma radiation, it was commonly observed that pre-yield (elastic) behavior of cortical bone tissue is unaffected while post-yield (plastic) properties suffer a significant reduction as a result of radiation sterilization [1,2,5,12,18,39]. Degradation in post-yield properties results in the loss of tissue ductility and gamma radiation sterilized bone tissue becomes brittle [1,2,5, 12,18,39]. This behavior is hypothetically explained by the dependency of pre-yield properties of cortical bone tissue on the mineral phase and the post-yield properties on the collagen [7,43]. Therefore, it is believed that the collagen phase is more vulnerable to gamma radiation than the mineral phase of cortical bone tissue. Besides gamma radiation induced embrittlement of bone, age [32,45] and disease [20,40] related alterations in collagen biochemistry have also been documented to cause bone brittleness. Thus, it is widely accepted that the integrity of bone's collagen profoundly effects the mechanical strength and fracture resistance of bone tissue [41].

The effect of gamma radiation on the biochemistry of collagen has also been extensively investigated for collagen extracted from tendons [3,9,34]. It is shown that gamma radiation leads to scission of the peptide backbone [3,9], and reduces the concentration of intermolecular cross-links in tendon collagen [34]. Similarly to tendon, destruction of the peptide backbone of bone's collagen [17] and reduction in intermolecular cross-link density [10] have been reported for human femoral cortical bone secondary to gamma radiation sterilization.

The majority of gamma radiation damage is induced by damaging species (i.e. free radicals) resulting from the radiolysis of water molecules [16,37]. In this regard, collagen is a primary target for radiation-based free radical attack due to the significant amount of water bound to its structure [36]. Free radicals resulting from the radiolysis of water react with target molecules within a lifetime on the order of 0.01–1 ns and render irrecoverable changes in the target molecule's chemical structure [16]. Particularly, the hydroxyl ($OH^{\cdot}$) free radical induces the greater portion of the in vivo and in vitro damage to biological systems during gamma radiation [19,37,42]. Supporting the existence of a water-based free radical attack, bone irradiated at −78 °C was less brittle and had less collagen damage than when irradiated at room temperature [17]. So as to explain this observation, Hamer et al. speculated that the mobility of water molecules is restrained in the frozen state and, in turn, fewer free radicals are generated [17]. It seems likely that gamma radiation impairs the structure of the collagen matrix by water-based free radical attack [10,17]. However, data supporting this assertion are scant.

The purpose of the current study was to minimize the mechanical impairment of gamma radiation sterilized bone tissue by limiting the free radical damage pathway to the collagen phase. The following experiments tested the hypothesis that human cortical bone tissue sterilized in the presence of the free radical scavenger thiourea will be mechanically superior (i.e. less brittle) to cortical bone tissue sterilized in the absence of thiourea. For this purpose specimens were subjected to monotonic and cyclic tensile tests to assess the potential of the free radical scavenger thiourea for reducing gamma radiation related impairment of bone. The radioprotective effect of thiourea on collagen was investigated qualitatively by SDS-PAGE analysis and by fractographic investigation of failure surfaces via SEM.

Materials and methods

Preparation of tensile test specimens

Femurs from three male cadavers (ages 31, 31, 38) were obtained from the Musculoskeletal Transplant Foundation (Jessup, PA, USA). The diaphyses were sectioned into two segments, each approximately 55 mm long, using a hacksaw. The first segment was taken immediately distal to the minor trochanter and the second distally from the first segment. A low-speed metallurgical saw with a diamond coated blade (SouthBay Tech, CA, USA) was used to cut the rings into the four anatomical quadrants. One millimeter thick wafers were sectioned from the quadrants within the circumferential-longitudinal plane (parallel to osteonal orientation) using the low-speed metallurgical saw. Wafers were then cut into beams with final dimensions of 40 mm × 5 mm × 1 mm. Coupon shaped tensile test specimens were machined from the beams by reducing the width of the mid-gage region using a table-top milling machine (Sherline, CA, USA) and a 0.5″ diameter end-mill. The gage region had the final dimensions of 2 mm × 1 mm × 16 mm. Specimens were kept wet with calcium supplemented saline solution by using an air-pressure driven nozzle spray during the milling process. Four treatment groups were included in the study: controls (C), thiourea treated controls (1.5C), irradiated (IR) and thiourea treated-irradiated (1.5IR). Specimens were randomly assigned to each of the four treatment groups. Fifteen monotonic and four fatigue specimens were assigned to each treatment group, reaching a total of 76 specimens. Specimens were kept wet at all times and stored at −40 °C.

Thiourea treatment and sterilization

Free radical scavengers are substances that inhibit free radical damage to a target molecule by direct chemical reaction with the radical or by minimizing the formation of the radical. Free radical damage to collagen extracted from tendons was previously inhibited by using the scavengers thiourea [3,25] and cysteamine [25]. Riedle and Kerjaschki