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Research Paper

Effects of high-dose gamma irradiation on tensile properties of human cortical bone: Comparison of different radioprotective treatment methods

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*Farzaneh Allaveisi, Majid Mirzaei**

Department of Mechanical Engineering, Tarbiat Modares University, Tehran, Iran

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ABSTRACT

There are growing interests in the radioprotective methods that can reduce the damaging effects of ionizing radiation on sterilized bone allografts. The aim of this study was to investigate the effects of 50 kGy (single dose, and fractionated) gamma irradiation, in presence and absence of L-Cysteine (LC) free radical scavenger, on tensile properties of human femoral cortical bone. A total of 48 standard tensile test specimens was prepared from diaphysis of femurs of three male cadavers (age: 52, 52, and 54 years). The specimens were assigned to six groups ($n=8$) according to different irradiation schemes, i.e.; Control (Non-irradiated), LC-treated control, a single dose of 50 kGy (sole irradiation), a single dose of 50 kGy in presence of LC, 10 fractions of 5 kGy (sole irradiation), and 10 fractions of 5 kGy in presence of LC. Uniaxial tensile tests were carried out to evaluate the variations in tensile properties of the specimens. Fractographic analysis was performed to examine the microstructural features of the fracture surfaces. The results of multivariate analysis showed that fractionation of the radiation dose, as well as the LC treatment of the 50 kGy irradiated specimens, significantly reduced the radiation-induced impairment of the tensile properties of the specimens ($P<0.05$). The fractographic observations were consistent with the mechanical test results. In summary, this study showed that the detrimental effects of gamma sterilization on tensile properties of human cortical bone can be substantially reduced by free radical scavenger treatment, dose fractionation, and the combined treatment of these two methods.

1. Introduction

In the last decades, there have been growing interests in bone allografts for replacements or reconstructions that are carried out in the treatments of traumatic injuries, tumor removal, and other bone diseases. However, there are limitations due to the possibility of disease transmission from donor to host (Nather et al., 2006). Thus, terminal sterilization of the allografts with high-dose irradiation is critical prior to their implantation. In particular, high-dose irradiation of the allografts with gamma ray and electron beam (Ebeam) is used as a standard method for neutralizing the microorganisms. In

*Corresponding author. Fax: +98 21 82884909.
E-mail addresses: mmirzaei@modares.ac.ir, majidmirzaei@hotmail.com (M. Mirzaei).

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practice, the D_{10} (kGy) is defined as a required dose to eliminate 90% of the microorganism population. This factor is used for evaluation of the effectiveness of sterilization via ionizing radiation (Kaminski et al., 2010). Although a minimum dose of 15 kGy is considered necessary to achieve a sterility assurance level (SAL), several reports have indicated that this level of gamma irradiation cannot be regarded as a significant virus inactivation method for bone allografts (Nguyen et al., 2007). On the other hand, it has been reported that complete sterilization of the radiation-resistive pathogens is achieved through radiation doses up to 70 kGy (Barth et al., 2011). Nevertheless, due to the concerns over the effects of radiation damage on the mechanical integrity of the bone tissue, there are questions regarding the efficiency of the routine irradiation of the bone allograft.

It should be noted that the mechanisms of these damages are related to the direct or indirect effects of gamma irradiation on the constituents and microstructure of the bone (Kaminski et al., 2010; Cheung et al., 1990; Bailey, 1967; Hamer et al., 1999). The direct effect leads to polypeptide chain breakage of dried collagen, whereas the indirect effect essentially occurs in wet bone (Kaminski et al., 2010). The indirect effect acts through successive events including the radiolysis of the bound water (BW), forming the highly reactive free radicals (principally the hydroxyl ($OH^{\bullet}$)), and inter- and intra-molecular cross-linking effects (Kaminski et al., 2010; Barth et al., 2011; Salehpour et al., 1995; Dziedzic-Goclawska et al., 2005). The free radicals, in turn, produce chain reactions by reacting with each other, tissue organic molecules, oxygen, and water tissue constituents (collagen, fibers, protein, enzyme, salts, minerals, and hydroxyapatite) (Nather et al., 2006). The main productions of these chain reactions are Hydrogen peroxide (H_2O_2), hydroperoxy radicals (HO_2^*), and organic peroxy radicals (RO^*_2), which inhibit DNA synthesis of living organisms and have detrimental effects (Nather et al., 2006; Hall and Giaccia, 2006).

Another important issue is that the bone mineral component is primarily responsible for elastic (pre-yield) properties of bone, while the organic component (mainly type I collagen fibrils) is responsible for its plastic (post-yield) behavior (Akkus et al., 2005; Akkus and Rimnac, 2001; Currey et al., 1997). The studies on detrimental effects of gamma sterilization on elastic and plastic properties of the bone have demonstrated that decreased cross-linking of the collagen (Cheung et al., 1990; Bailey, 1967; Salehpour et al., 1995; Akkus et al., 2005; Ward, 1990; Wang et al., 2001; Colwell et al., 1996) significantly affects the mechanical properties of the bone within the plastic region (Akkus et al., 2005; Currey et al., 1997; Hamer et al., 1996) and results in ductility reduction, while elastic properties remain almost unaffected (Akkus et al., 2005; Currey et al., 1997). For instance, the reduction of the post-yield strength (by 70–87%) and decrease of the fatigue strength (by 87%) of the femoral cortical bone exposed to 36.4 kGy have been reported (Akkus et al., 2005; Currey et al., 1997). Mitchell et al. (2004) employed the subcritical cyclic loading stress and showed that sterilization via 31.7 kGy gamma irradiation leads to significant reduction in the resistance of the human cortical bone to fatigue crack propagation. They concluded that the reduction of the bone ductility and toughness was related to degradation of the collagen matrix following ionizing radiation. Islam et al. (2015) investigated the effect of gamma radiation sterilization on the high-cycle fatigue life of allograft bone. They observed that gamma sterilization severely impaired the high cycle fatigue life of structural allograft bone tissues. They emphasized that the impairment was stronger than those reported for monotonic mechanical properties, and suggested that research on methods to reduce these effects are important to pursue. Barth et al. (2011) investigated the effects of x-ray irradiation on the hierarchical structure and mechanical properties of the hydrated human cortical bone. They reported a dose-dependent trend in the impairment of the bone strength, ductility, and fracture resistance following exposure to 0.05–630 kGy. The irradiation-induced detriments were attributed to alterations in the mechanisms of the different length-scales of the bone, including micron-scales (variations in crack paths), sub-micron levels (stiffening and degradation of the collagen due to suppressing the fibrillar sliding), and nano-scales (loss of the collagen because of the collagen matrix impairment).

The results of these studies show that the improvement of the collagen integrity is of utmost importance in prevention of the degradations of mechanical properties following ionizing sterilization.

It has also been shown that the treatment of the bone and tendon allografts during gamma sterilization by free radical scavengers such as thiourea (Akkus et al., 2005), riboflavin (Seto et al., 2012), and ascorbate (Seto et al., 2012) can alleviate the collagen detriments and improve mechanical integrity. Table 1 shows different methods of allograft radioprotection that have been used based on the capture or reduction of the irradiation-induced free radicals prior to their attack to the collagen matrix.

According to Seto et al. (2012), free radical scavenger treatment combined with crosslinking maintain the primary mechanical properties of the 50 kGy gamma sterilized tendons. Another attempt to reduce the radiation-induced damages has been reported by Hoburg et al. (2011). They used the fractionation of 34 kGy E-beam irradiation (3.4 kGy at 10 fractions) for improving the viscoelastic and structural properties of bone-patellar tendon-bone (BPTB) grafts. Furthermore, Wei et al. (2013) showed that splitting the sterilization dose of 50 kGy to 20 equal fractions maintains the native post-yield tensile properties of the human flexor digitorum superficialis tendons.

However, the free radical scavenger treatment may compromise the sterility by protecting pathogens. In this regard, Shuster (2005) suggested that the proper scavenger should allow for the protection of the collagen structure of the allograft tissue but not the pathogens during irradiation. In another study, Kattaya et al. (2008) investigated some cysteine-derived radioprotectants which can efficiently perfuse bone yet minimally protect pathogens. They showed that LC radioprotectant does not shield bacterial spores against gamma radiation and may be suitable for curbing the radiation damage to bone grafts while achieving sterility. They suggested that further biomechanical experiments will be essential to assess the effects of LC on irradiated grafts.

Furthermore, the previous studies indicated that treating the bovine femoral cortical bone by N-acetyl-L-cysteine (NAC)