Radioprotection of Bone Allograft using Vitamin E Derivatives

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Statement of Purpose: Bone allografts are used for reconstruction due to illness, injury, or tumor removal. Terminal sterilization by chemicals or ionizing radiation is used against viral and bacterial disease transmission [1], which is of concern especially for Hepatitis and HIV [2]. Radiation sterilization impairs the mechanical strength of bone, particularly the bending strength, work to fracture, and impact energy [3]. This decrease is likely the cause of an increased incidence of fracture in irradiated bone allografts (39 vs. 18%; [4]).

We hypothesized that radioprotection of allograft bone could be achieved by infusing with the naturally occurring antioxidant vitamin E prior to irradiation to improve the fracture toughness, work to failure, and IZOD impact strength of irradiated bone.

Methods: Sample preparation: A total of four bovine tibias were obtained from Animal Technologies. Two tibias were used for testing under bending (Bone 1 and 2) and the other two (Bone 3 and 4) for IZOD impact testing. For bending, the diaphysis of the tibias was machined to 3.7x3.7x55 mm blocks. For impact, the tibias were machined to 4x10x50 mm. Three different types of treatment were used: (1) no treatment, (2) irradiation only at 25 kGy, (3) infusion with vitamin E or a derivative followed by irradiation at 25 kGy. Samples were then stored at -20^{o}C until testing.

Mechanical Testing: Prior to testing, samples were soaked in PBS at room temperature for at least 30 minutes. For bending, samples were notched to 1 mm depth. Three-point bending tests were conducted using Insight 2 (MTS) at a rate of 10 mm/min. Fracture toughness and work-to-failure were calculated according to ASTM C1421-10. For the IZOD impact test, samples were also notched to 1 mm depth and tested according to ASTM F658-07 using Instron CEAST 9050. Recovered properties were calculated as: (Value of treated and irradiated samples-Value of irradiated only samples)/(Value of untreated samples-Value of irradiated only samples) * 100 %.

Reflection-Based FTIR: Bone blocks (n=3 from each) were polished sequentially with 600 grit, 800 grit and 1200 grit carbide paper for 3 minutes each. Collagen cross-linking was analyzed using a reported method [5] using specular reflection geometry at a resolution of 4 cm^{-1} and 150 scans for each spot. Spectra were analyzed using the Kramers-Kronig relationship [5]. A collagen cross-linking index was determined by using the ratio of peaks at (1661-1659 cm^{-1})/(1691-1689 cm^{-1}) [6,7].

Results: Irradiation of bone 1 caused the fracture toughness (FT) to decrease by 57 ± 1.0 % and work to failure (WF) by 69 ± 3.0 % as compared to untreated bone. Infusing bone 1 with vitamin E prior to irradiation resulted in FT recovery of 36% (95 % CI: 23 -48 %) and WF recovery of 40 % (95 % CI: 3.7 -77 %). Irradiation of bone 3 caused the IZOD impact strength to decrease by 47± 9.5 %. Infusing bone 3 with vitamin E prior to irradiation resulted in statistically significant recovery of the IZOD impact strength by 39 % (95 % CI: 8.2%-69 %).

Irradiation of bone 2 decreased the FT by 39 ±2.0 % and WF by 73 ±1.0 %. Infusing bone 2 with vitamin E derivative prior to irradiation resulted in FT recovery of 60 % (95% CI: 22-99 %) and WF by 52 % (95 % CI: 32-71 %). Irradiation of bone 4 caused the IZOD impact strength to decrease by 39 ± 15 %. Infusing bone 4 with vitamin E derivative prior to irradiation resulted in recovery of the IZOD impact strength by 91 % (95 % CI: 66-100 %).

Irradiation resulted in a decrease of 57 ±2% in the collagen crosslinking index (Fig 1). Infusing bone 2 with vitamin E derivative prior to irradiation resulted in collagen crosslinking index ratio recovery by 75 % (95 % CI: 71-79 %).

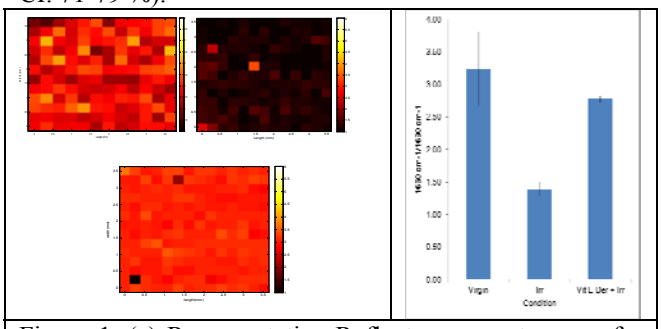

Figure 1. (a) Representative Reflectance spectra map for each treatment. (b) Average 1660/1690 cm^{-1} for different treatments.

Conclusions: Our hypothesis that radioprotection of allograft bone could be achieved by infusing it with vitamin E tested positive. We showed that infusion by vitamin E or its derivative enabled significant recovery of the mechanical properties detrimentally affected by irraidation. Treatment protected collagen crosslinking, the deterioration of which is the major mechanism behind the effects on the mechanical properties of bone allograft.

One limitation of our study is the variation in the recovery of the measured mechanical properties, presumably due to the lack of control over bone composition. It is known that bovine bone contains different percentage of fat, calcium, and collagen as a function of age [6,7].

Improving the mechanical properties of bone allograft may benefit the load-bearing capability of structural grafts used in skeletal reconstruction as well as that of morselized grafts used in spinal fusion.

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