How to toughen irradiation-sterilized bone allograft

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Introduction: Bone embrittlement due to γ-irradiation sterilization is a concern for tissue banks (product quality) and for orthopaedic surgeons (clinical effectiveness and outcomes). This is especially true in cases of large structural allografts (massive intercalary defect reconstruction) because only 15-20% of such a graft will be invaded and remodeled by host cells (5y; host-graft junction) [1]. The rest of the graft remains dead and yet must tolerate damage due to millions of cycles of loading per year for many years. Irradiation-sterilized bone is thought to become brittle and less damage tolerant because the collagen becomes fractured [2] and connectivity is lost. Such bone acts like a brittle ceramic with greatly reduced toughness [3], fracture toughness [4,5], fatigue resistance [6] and altered microdamage formation [3]. Various alternatives have included avoiding irradiation altogether, switching to chemical sterilization or irradiating in the presence of radio-protectants [2]. Our novel approach consists of working with the irradiation to allow effective sterilization while providing a new toughening phase made of in situ modified collagen. Based on the knowledge that irradiation degrades collagen connectivity, we hypothesized that irradiation-driven glyco-oxidation crosslinking (GOC) would help provide toughening to irradiation-sterilized bone allograft. ***Objectives:*** (1) Evaluate our treatment as a method to improve irradiation-sterilized bone allograft toughness (2) Study changes in the bone collagen as a result of irradiation with/without our novel treatment method in order to gain insights into the mechanisms at play.

Methods: The experiments reported within used cortical bone from the distal half of bovine (steers 1-2 y old) tibia diaphysis. From each site, three matched beams (one set) were cut and polished to a 1-um finish. One beam from each set was randomly assigned to one of three groups: non-irradiated controls (N; kept frozen until testing), Irradiated controls (I), and the GOC test group. The I and GOC groups were incubated in physiological buffer. The GOC agent was ribose. The I and GOC groups were then packed in dry ice and irradiated at 30kGy. All specimens were then thawed and rehydrated in PBS before testing. ***Experiment A***: 20 sets were used. The beams were 2x4x60mm. All beams were scanned with DEXA to measure bone mineral density (BMD). The beams were tested to failure in three-point bending (ASTM D790). ***Experiment B***: 10 sets were used. The specimens were 4x4x60mm single edge notch bend (SENB) fracture specimens (ASTM E1820) and notched using a diamond wire saw followed by a razor with diamond paste. Initial notch length (a0) and crack length at instability (ai) were measured using scanning electron microscopy in order to estimate fracture toughness (K, J) at crack initiation and instability. ***Experiment C***: 10 sets of beams that had been tested to failure in three-point bending were demineralized in EDTA to obtain the organic component of the bone. Hydrothermal Isometric Tension (HIT) tests were performed on the collagen to evaluate thermal stability and connectivity. SDS-PAGE gel electrophoresis of pepsin-digested bone collagen (pepsin liberates the alpha chains) was run on a 4-20% gradient gel against a reference to compare alpha and beta chain content (band intensity) and extent of fragmentation (smearing). Statistical Analyses: Repeated measures ANOVA with Holms-Sidak tests post-hoc were used to test between group differences at the 95% confidence level.

Results: ***Experiment A:*** The I group had reduced ultimate strength (US), failure strain, damage fraction and work to fracture (WFx) but not modulus or yield strength. GOC resulted in notable improvements of the affected measures in I. US returned to N levels and GOC recovered 58% of the WFx lost in I ($p<0.001$). BMD was not detectably different between the groups. ***Experiment B:*** All measures of fracture toughness were reduced in I relative to N ($p<0.001$). The GOC group demonstrated notably improved fracture toughness (47% recovery; $p=0.043$) and work-to-fracture (42% recovery; $p=0.044$). See figure. ***Experiment C:*** HIT testing for I showed a 44% decrease in maximum isometric force ($p<0.005$) which reflects loss of integrity of the collagen network. The GOC group demonstrated 52% recovery of maximum isometric force ($p=0.027$). Gel electrophoresis showed less defined alpha bands, absence of the beta-band and smearing in the irradiated and GOC treated beams. This indicates alpha-chain fragmentation, loss of connectivity from native crosslinking (beta), and heterogeneous fragmentation (smearing).

Discussion: These data suggest that the in situ modified collagen phase created by irradiation-driven GOC during sterilization may be an effective means of improving the mechanical performance of irradiation-sterilized allograft bone. This is somewhat surprising because increased crosslinking in normal bone is thought to lead to reduced toughness. Data from Experiment C suggests that the new collagenous phase in our GOC treated specimens is more connected than in the I group and that a significant relationship between the connectivity of the collagen network and the toughness of bone allograft may exist. To our knowledge, this is the first study to demonstrate that the mechanical properties of irradiation-sterilized bone allograft can be improved using GOC during irradiation. This suggests that improved graft quality and clinical outcomes for large structural allograft applications are possible.

References: [1] Enneking, JBJS, 2001 [2] Akkus, JOR, 2005[3] Currey, JOR, 1997 [4] Akkus, JOR, 2001 [5] Barth, Biomats., 2011 [6] Akkus, JOR, 2005

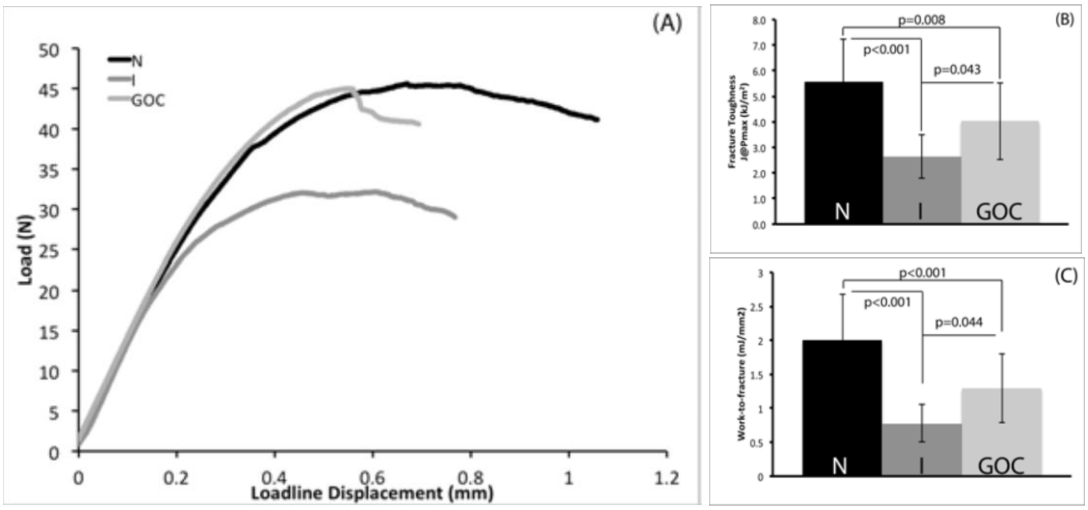

Figure: (A) Example curves from SENB fracture tests. (B) Fracture toughness data (J @ P_{max}, N/mm). (C) Work-to-fracture data (mJ/mm^2)