COMPARISON OF THE BIOMECHANICAL PROPERTIES OF NON-GAMMA IRRADIATED AND GAMMA IRRADIATED DENSE CANCELLOUS BONE

Jacob Wisbeck, MD
Benjamin Majors, MS
Laura Zagrocki Brinker, DPM
Disclosures

• Benjamin Majors, MS and Laura Zagrocki Brinker, DPM are paid employees of Paragon 28, Inc. with stock or stock options with Paragon 28, Inc.

• Jacob Wisbeck, MD is a paid consultant of Paragon 28, Inc.

• Processing and testing of specimens was performed at Community Tissue Services in Dayton, OH.
Background

- Allograft bone is used for a myriad of purposes
- Variations in processing between tissue banks exist
  - **Hydrogen peroxide**
    - Assists in cleaning and sterilization of bone graft\(^1\)
    - Has not been shown to affect the mechanical properties, but has a statistically significant effect on osteoinductivity, showing a linear decrease with increasing peroxide soak time\(^2\)
  - **Ethylene Oxide**
    - Terminal sterilization technique shown to be virucidal, but is harmful to osteoinductivity\(^2, 3\)
  - **Gamma irradiation**
    - Terminal sterilization technique\(^2\)
    - 19 fold fatigue life reduction at 35 MPa during high cycle fatigue in gamma irradiated samples (25-27 kGy) as compared to control (non-gamma irradiated) samples\(^3\)
  - **Aseptic**
    - Absent of gamma irradiation, hydrogen peroxide and ethylene oxide sterilization techniques
- Variations in processing may create differences in biomechanical properties of dense cancellous bone
Purpose

• Determine if using gamma irradiation during processing will significantly decrease the modulus, maximum compression stress and strain and energy during static testing of dense cancellous bone

• Determine if using gamma irradiation during processing will significantly decrease the strain energy of the allograft during dynamic testing in dense cancellous bone

• This is a pilot study to determine the above two objectives
Methods – Set Up

• Study approval per Community Tissue Services, an AATB accredited tissue bank
• Permission was received to acquire and use 2 research donors
  • Male, age 18
  • Male, age 36

Specimen Preparation
• Remaining muscle and other soft tissue were removed from the lower extremity
• A band saw was used to remove cartilage and cortical bone (Fig. 1)
• Test sites included: femoral head, femoral condyle and proximal tibia
• An 8 mm hole saw was used to create dowels that were then cut to a length of 8 mm (Fig. 2)
• Processing steps consisted of a series of static soaks (saline, antibiotics, isopropyl alcohol) and freeze drying for all specimens
  • Right side specimens were kept as aseptically processed donors
  • Left side specimens were sent for gamma irradiation (12.7-13 kGy)
Methods – Static Testing

• All samples re-hydrated in sterile saline for a minimum of 5 minutes
• N=36 samples
  • 18 aseptically processed
  • 18 low-dose gamma irradiated
• Measurements were taken for all samples prior to testing
  • Height
  • Weight
  • Diameter
  • Volume and density calculated from height/weight/diameter measurements

• All tests ran on Instron E3000 (3kN load cell)
  • Ramp displacement was set to 1 mm/min until maximum displacement of 3 mm was reached
  • Ultimate failure was defined at 10% strain based on similarly designed studies
  • Bluehill 3 ver 3.61 statistical software was used to analyze the static testing data
Methods – Dynamic Testing

- All samples re-hydrated in sterile saline for a minimum of 5 minutes
  - N=36 samples
    - 18 aseptically processed
    - 18 low-dose gamma irradiated
  - Identical measurements were taken as the static samples

- All samples re-hydrated in sterile saline for a minimum of 5 minutes and were kept constantly hydrated in saline during testing

- All tests ran on Instron E3000 (3kN load cell)
  - Initial displacement performed at 1 N/s up to 7 N compression
  - Sinusoidal load waveform set
    - 27 N amplitude, ranging from 7N to 61 N at 2 Hz
      - Low load based on static testing results
      - 10,000 cycle runout (approximately 90 minutes)
  - WaveMatrix ver 1.8 statistical software was used to analyze the dynamic testing data
Results – Static Testing

Energy – Toughness

Area under the stress strain curve is a measure of energy and is defined as toughness. It represents the ability of a material to plastically (permanently deform) without fracturing or failing.

To be tough, a material must withstand both high stress and high strains. A measure of impact resistance.
Results – Dynamic Testing

- 36% reduction in Cumulative Energy at 1000th Cycle
  - P = .02

- 37% reduction in Cumulative Energy at 2500th Cycle
  - P = .03

- 39% reduction in Cumulative Energy at 5000th Cycle
  - P = .05

- Six (6) samples in the irradiated treatment group failed before 10,000 cycles.
- One (1) sample in the aseptic treatment group failed before 10,000 cycles.
Discussion

• Aseptic processing has been shown in the literature to preserve osteoinductivity of allografts as well as contributing to maintenance of structural properties of allograft bone.

• This pilot study confirms previous studies using cortical bone that gamma irradiation degrades the structural properties of cancellous allograft bone.

• Statistically significant differences in cumulative energy absorption were observed between treatment groups during dynamic testing. Energy absorption decreased by 36-39% in the irradiated group.

• Differences in the average between the two treatment groups were observed for all static material properties, though not statistically significant: modulus, max stress, strain at max stress, and energy. The most drastic difference was observed in energy (37% reduction in irradiated group).
Discussion

• The irradiated treatment group showed significantly less ability to absorb energy in both static and dynamic testing. Further, six (6) irradiated samples could not complete dynamic testing. The results demonstrate that gamma sterilization of cancellous allograft increased vulnerability to impact loading and fatigue crack resistance.

• The study is not without limitation
  • Pilot study = small sample size
    • Further research in this topic is warranted with a larger sample population
  • Large standard deviations prevented finding statistical differences between the two treatment groups for certain material properties
    • Orientation of bone dowel harvest was not consistent between treatment groups
    • The anisotropy of bone may have contributed to variation in sample data and thus resulted in higher standard deviations
    • Harvest site location may have contributed to differences in material properties
    • Variability may exist between donors
References


