Repeated Freeze-thaw Cycles Do Not Alter the Biomechanical Properties of Fibular Allograft Bone

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Introduction

Structural allograft bone is commonly used in orthopaedic surgery for treating non-unions, spanning bone defects, peri-prosthetic fractures, tumor reconstruction, arthrodesis, and spine surgery. Complications relating to unanticipated graft fracture have prompted investigation into possible sources of graft failure, including storage and handling conditions. Allograft bone is typically frozen at temperatures ≤-40 °C and then thawed prior to clinical use. Since there are no regulations or guidelines regarding this process, grafts may be subjected to multiple freeze-thaw cycles at both the tissue bank and surgery center. The objective of this study was to determine the effects of freeze-thaw cycles on the biomechanical properties and structural integrity of fibular allograft bone. Our hypothesis was that the mechanical properties of fibular allograft bone decrease with increasing numbers of freeze-thaw cycles.

Methods

Left and right fibulas were recovered from 18 research-consented cadaveric donors (seven males and eleven females, 39-75 yrs old). Each fibula was thawed, debrided, cut into three 80mm segments, and subjected to the Allowash® bioburden reduction process. Since a minimum of one freeze-thaw cycle is incurred between tissue recovery and processing, one segment was defined as the control and immediately tested. The remaining segments were randomly assigned to one of the five treatment groups: two, four, or eight freeze-thaw cycles (2FT, 4FT, 8FT), one freeze-thaw cycle plus freeze drying (1FT-FD), or three freeze-thaw cycles plus freeze-drying (3FT-FD). A freeze-thaw cycle is defined by the American Association of Tissue Banks standards as a tissue being frozen (at a temperature of -40°C or colder) and then allowed to warm until the residual water undergoes the solid to liquid phase change (~1-2 °C). Freeze-drying was conducted according to validated protocols to result in a moisture content < 6% by weight.

After the assigned number of freeze-thaw cycles was achieved, each specimen was imaged in a CT scanner (Stratec XCT-2000). CT slices were obtained at three locations about the mid-point of the segment length, at the mid-point and ±4 mm of it. The segment was placed on a radio-transparent fixture with the same configuration as the bending fixture and in the same orientation as it would be placed for testing. The measurements (i.e. areal moment of inertia, cortical thickness, and neutral axis dimension) used for the analysis were averaged over the three slices. Prior to testing, 5mm sections were removed from each segment and placed in 10% buffered formalin for histological analysis. Specimens were tested in three-point bending by loading to failure at a strain rate of 0.5 mm/sec on a materials testing system (Instron E3000).

Structural parameters (load, stiffness) were measured, from which material parameters (stress, strain) were derived using specimen dimensions obtained from the CT data. Stiffness and modulus were calculated as the slope of the force displacement curve and stress strain curve, respectively, between 20% and 80% of the peak force or stress. Stress and strain were calculated as:

$$\sigma = \frac{F \gamma_x}{4L} \quad \epsilon = \frac{\Delta L}{L_o}$$

where F = measured load, L = span between anvils (50mm), \(\gamma_x\) = maximum distance from cortical edge to horizontal axis, \(L_o\) = areal moment of inertia about the horizontal axis, \(\Delta\) = measured deflection, \(L_o\) = cortical thickness. Energy was calculated by integrating the force-displacement curve up to the point of failure.

A two-way ANOVA was used to test for significant differences between donors and/or treatments, and a post-hoc Tukey’s test for pairwise treatment mean comparisons. Correlation coefficients were calculated for the aforementioned measures along with donor age and segment location within the bone.

Results

The average ultimate stress for all segments was 174±59 MPa, average modulus was 289±217 MPa, average energy was 2.00±1.83 J, and the average stiffness for all segments was 1320±511 N/mm. There were no significant differences between treatment groups for these measures (Figs 2 and 3). However, post-hoc analysis revealed a significant difference (p<0.05) between 1FT-FD and Control and 2FT and Control in stiffness and energy, respectively. Donor variation was significant for all measures at p<0.001. All reported quantities were negatively correlated to donor age, but no correlation exists for segment location.

Conclusions

The results from this study suggest that there is no effect on the failure properties of fibular bone segments when subjected to as many as eight freeze-thaw cycles and/or freeze-drying. Furthermore, there is no detectable trend of property change as the number of cycles increase. However, it is apparent from the data that the donor-to-donor variation is quite large. The post-hoc test of means showed no difference between the control and freeze-dried treatments in terms of energy, but inspection of Fig 3 and the loading profile (not shown) implies that the sub-failure fatigue properties of these groups would be markedly different. Moreover, the post-hoc test did indicate a difference between the freeze-dried and the 2FT, 4FT, and 8FT treatments, which implies that freeze-dried bone warrants further study in the sub-failure region.