Gamma Irradiated Soft Tissue Allografts are Biomechanically Equivalent to Aseptic, Non-Sterilized Tissues

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Abstract

Allograft safety is contingent on effective sterilization methods such as gamma irradiation. However, recent clinical studies have associated gamma irradiation with decreased graft strength and increased allograft failure rates, despite data that shows no deleterious effects to soft tissue biomechanics at gamma doses between 1.46 and 2.18 mRad. The objective of this study was to compare the biomechanical properties of anterior and posterior tibialis tendons and bone-patellar ligament-bone (BTB) allografts sterilized with gamma irradiation at a dose of 1.7-2.1 mRad to aseptically processed, non-irradiated tendons. Our hypothesis was that gamma irradiated tendons would be biomechanically equivalent to aseptic controls. Pairs of fresh-frozen tibialis tendons and bisected BTBs were either gamma sterilized or left untreated to serve as aseptically processed, non-sterilized controls. Each graft was cyclically loaded in tension for 2000 cycles at a rate of 2 Hz, allowed to relax for five minutes and then tested in tension to failure at 100% strain rate. Gamma irradiated BTBs exhibited structural (maximum load, maximum displacement, stiffness) and material (failure stress, failure strain, and elastic moduli and cyclic tendon elongation) properties that were not significantly different from non-sterilized controls. Gamma sterilized tibialis tendons were not significantly different from aseptic controls for the three structural properties and three of the four material properties, but did have lower cyclic elongation compared to controls. This difference suggests that gamma-treated tibialis tendons may be less susceptible to laxity than aseptic grafts. This study reinforces that tendon sterilization via gamma irradiation yields a safe allograft without comprising biomechanical function.

Introduction

Gamma irradiation has an extensive history of use for sterilizing soft tissue allografts. Early research showing dose-dependent reductions in musculoskeletal tissue biomechanics at high gamma doses (≥ 3.0 mRad)[1-3] has prompted tissue banks to employ lower doses that remain extremely efficient in deactivating microorganisms while minimizing tissue damage. Recent studies have shown that low dose gamma irradiation, loosely defined as approximately 1.5-2.0 mRad, does not alter the biomechanical properties of bone-patellar tendon-bone (BTB), tibialis, and semitendinosus tendon allografts[4-5]. Still, clinical studies reporting higher failure rates in ACL reconstructions using allografts, especially in younger patients[6-8], have implicated gamma irradiation as a primary cause for failure without a full understanding of potential failure mechanisms[9-10]. This negative perception has spurred increased use of aseptic grafts to safeguard biomechanical integrity, but it comes at the risk of implanting a non-sterile graft. Further research is needed to support gamma irradiation use at appropriate doses that do not affect the graft's biomechanical integrity.

> ***The purpose of this study was to compare the biomechanical properties of tibialis tendons and BTBs sterilized with gamma irradiation to aseptic tendons.***

Study Objective

The purpose of this study was to compare the biomechanical properties of anterior/posterior tibialis tendons and BTBs sterilized with gamma irradiation at a dose of 1.7-2.1 mRad to aseptically processed, non-irradiated tendons. Our hypothesis was that gamma irradiated tendons would be biomechanically equivalent to aseptic controls.

Specimen Preparation

Paired, bisected BTBs from a group of 10 donors (6 male and 4 female, mean age 57.8 years, age range 49-72 years old) and paired anterior and posterior tibialis tendons from a different group of 10 donors (5 male and 5 female, mean age 65.1 years, age range 40-74 years old) were aseptically recovered and processed as fresh-frozen grafts per current standard manufacturing procedures that included a proprietary method that has been validated to a Sterility Assurance Level (SAL) of 10^{-6}. Tendons were randomly selected and either gamma irradiated on dry ice at a 1.7-2.1 mRad dose or left untreated to serve as aseptic controls.

Biomechanical Testing

Tissues were stored at ≤-65°C until testing, at which time they were thawed in a water bath set to physiologic temperature (37°C). All specimens were trimmed to a minimum gage length-to-width ratio of 3:1, and the cross-sectional dimensions and initial gage lengths were measured in the unloaded state using a laser micrometer and calipers, respectively. For BTBs, the tibial bone blocks were potted using Bondo® body filler resin in custom molds. For tibialis tendons, the ends were folded in sandpaper to prevent slippage. Both ends of each specimen were secured in pneumatic clamps and testing was conducted using a materials testing system (Instron ElectroPuls E3000; Norwood, MA). Each tendon was preconditioned via cyclic tensile loading from 0-20 N at 0.5 Hz for 10 cycles. The grafts were then subjected to 2000 cycles of cyclic tensile loading at a rate of 2 Hz, with loads sinusoidally ramped between 0 N and 200 N in load control[11]. Specimens were unloaded and allowed to relax for five minutes following the cyclic sub-failure testing. Lastly, the specimens were loaded in tension to failure at a displacement rate of 100% initial gage length per second[12-13]. All testing was conducted in a custom environmental chamber that maintained physiologic temperature (37±2°C) and constantly misted the specimens with saline to maintain tissue hydration

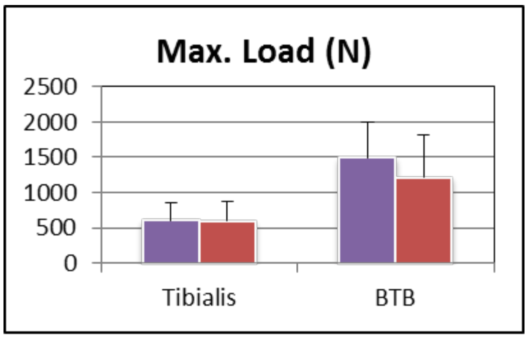
Data Analysis

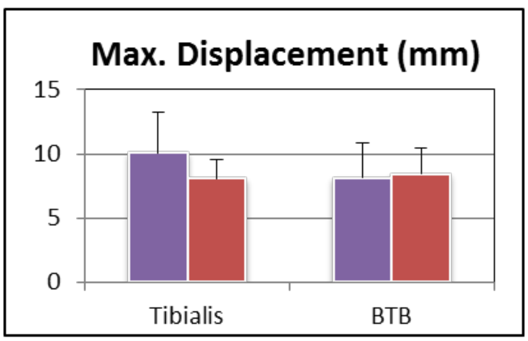
Total tendon elongation for the cyclic test was expressed as a percentage and defined as the strain difference following completion of the sub-failure loading. Structural and material properties were calculated from failure load-displacement curves and stress-strain curves, respectively. Nominal strain was determined from the ratio of grip-to-grip displacement to the initial specimen length. Stiffness was calculated as the slope of the linear portion of the load-displacement curves, typically defined between 20%–80% of the absolute peak force. Similarly, the elastic modulus was calculated from the stress-strain curves of the failure tests. Maximum load, maximum displacement, failure stress, and failure strain were all defined at the breaking point on the failure test curves. Mean and standard deviation were calculated for each biomechanical property, and a student t-test was used to detect differences between gamma irradiated and aseptically prepared tendons, and all reported p-values are two-tailed with $\alpha=0.05$ significance level.

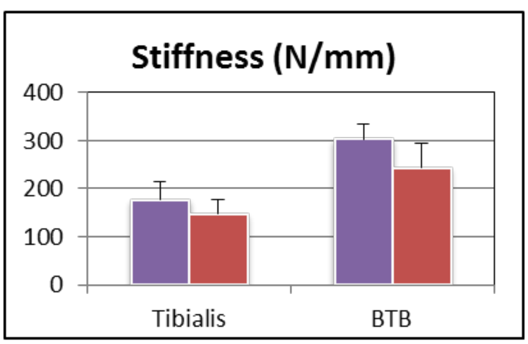
Results

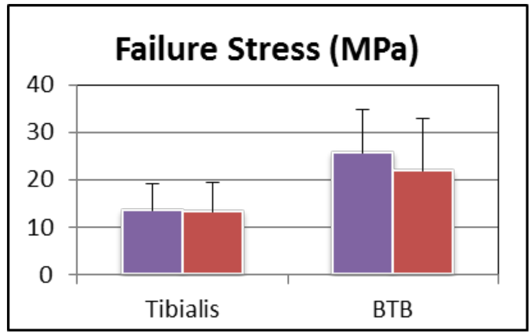
Gamma irradiated tibialis tendons exhibited structural properties (maximum load ($p=0.918$), maximum displacement ($p=0.737$), and stiffness ($p=0.053$)) and material properties (failure stress ($p=0.996$), failure strain ($p=0.716$), and elastic modulus ($p=0.196$)) that were not significantly different from aseptic controls. Tibialis tendons had significantly lower elongation after sub-failure loading compared to aseptic controls ($p=0.026$). Two tibialis tendons (one per treatment) failed during cyclic testing and were excluded from the failure analysis.

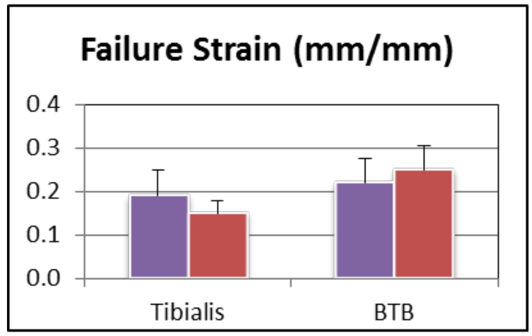
For gamma sterilized BTBs, the structural properties (maximum load ($p=0.473$), maximum displacement ($p=0.911$), and stiffness ($p=0.596$)) and material properties (failure stress ($p=0.667$), failure strain ($p=0.478$), and elastic modulus ($p=0.275$), and cyclic elongation ($p=0.310$)) were not significantly different from aseptic controls.

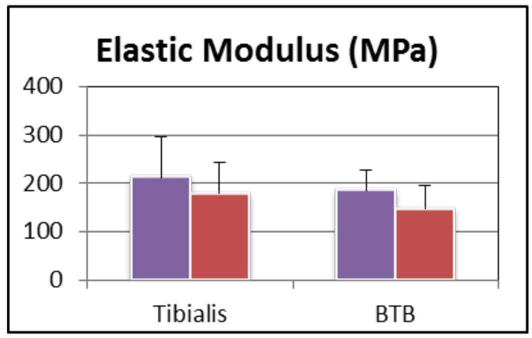


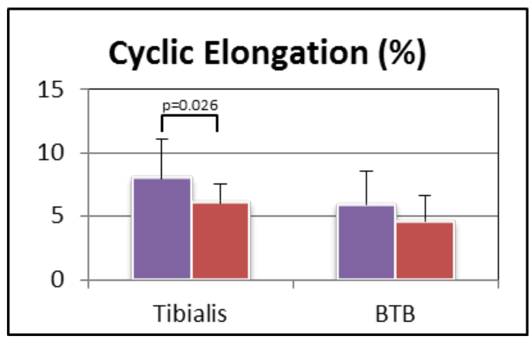












Conclusions

Patient safety is paramount, and our results support the use of gamma irradiation at doses of 1.7–2.1 mRad to provide safe soft tissue allografts that maintain similar biomechanical properties compared to non-irradiated, aseptic grafts. The observed reduction in irradiated tibialis elongation after cyclic loading suggests that the gamma-sterilized tendon may be less susceptible to clinical laxity compared to aseptic tibialis grafts.

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