Mechanical Properties of Tendon Allografts Following Low-Dose Radiation Exposure Prior to Processing

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Jeffrey Cartmell, Ph.D., Lead Scientist
Musculoskeletal Transplant Foundation
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SUMMARY

The effects of Post-Treatment gamma radiation (at varying dose levels) upon baseline mechanical properties of tendon allografts have been well documented,[1,2] however no such characterization exists for tendon allografts exposed to low-dose gamma radiation as a Pre-Treatment step (prior to processing).

Our hypothesis is that Pre-Treatment of allograft tendons with low-dose (15 kGy) gamma radiation will have no measurable effect upon baseline mechanical properties of human gracilis tendon and bone-patellar tendon-bone segments compared to donor-matched, non-gamma irradiated controls.

Human gracilis tendons and bone-patellar tendon-bone segments were randomly divided into Control (0 kGy) and Pre-Treatment (15 kGy target dose) groups. Donor-matched tendons (N=10) were used for all testing. Tendons in the Pre-Treatment group were exposed to 15 kGy of gamma radiation on dry ice at the bulk stage prior to processing. Tendons in the Control group were not exposed to any gamma radiation. All tendons were then processed into finished grafts using standard MTF procedures and stored frozen (-70ºC). Tendons were thawed overnight, cross-sectional area was measured and then the tendons were subjected to tensile testing to failure.

There was no difference in cross-sectional area, structural properties or mechanical properties between the Pre-Treatment and Control groups for either the gracilis tendons or the bone-patellar tendon-bone samples. Visually, tendons showed no noticeable differences between the Control and Pre-Treatment groups.

The data supports the hypothesis by demonstrating that Pre-Treatment of allograft tendons with low-dose gamma radiation (15 kGy target) has no measurable effect upon baseline mechanical properties of gracilis tendons or bone-patellar tendon bone segments. Therefore, we can conclude that: ***Pre-Treatment of allograft tendons with low-dose (15 kGy) gamma radiation has no measurable effect on mechanical properties.***

INTRODUCTION & BACKGROUND

Decontamination of allograft tissue may be accomplished via exposure to low-dose gamma radiation either prior to processing (Pre-Treatment radiation) or as a finished graft (Post-Treatment radiation). Typically, the dose range for the low dose gamma radiation exposure is 12 – 18 kGy, with a median target of 15 kGy. Pre-Treatment radiation exposure is so named because this treatment occurs with the allograft donor tissue still in its whole form prior to any dissections, cleaning or disinfection procedures. Post-Treatment radiation exposure occurs after all tissue (e.g., bone attachments, muscle, adipose, etc.) has been dissected, and the allograft tissue has been cleaned, disinfected and trimmed to create the finished graft. The effects of Post-Treatment gamma radiation (at varying dose levels) upon baseline mechanical properties of tendon allografts have been well documented,[1,2] however no such characterization exists for tendon allografts exposed to low-dose gamma radiation as a Pre-Treatment step.

Our hypothesis is that Pre-Treatment of allograft tendons with low-dose (15 kGy) gamma radiation will have no measurable effect upon baseline mechanical properties. This study used *in vitro* mechanical testing of human gracilis tendon (GT) and bone-patellar tendon-bone (BTB) segments to examine the baseline mechanical properties of allograft tendons pre-treated with low-dose gamma radiation (prior to processing) and compared the results to donor-matched, non-gamma irradiated controls. Gracilis tendons were selected to represent the range of shoestring tendons (without bone blocks) that are clinically available for anterior cruciate ligament reconstruction (ACLR), while BTB's were selected to represent tendons with bone blocks clinically available for ACLR.

METHODS

Human GT's and BTB's were randomly divided into two groups: Control (0 kGy) and Pre-Treatment (15 kGy target dose). Donor-matched tendons (N=10) were used for all testing, but separate donors were used for the GT and BTB specimens. Tendons in the Pre-Treatment group were exposed to 15 kGy of gamma radiation on dry ice prior to processing. In this state, allograft donor tendon tissue still has muscle and adipose attached and no tissue excision or debridement had yet occurred. Tendons in the Control group were not exposed to any gamma radiation. All tendons (from both groups) were then processed into finished grafts using standard MTF procedures including antibiotic soaks, soft tissue debridement, and trimming to size, then wrapped in saline-soaked gauze and stored frozen (-70ºC) until testing. The procedures utilized to clean and process the allograft tendons for this study are identical to those used by MTF in the preparation of allografts for clinical use.

Following overnight thaw (at 4 ºC), cross-sectional area (XSA) was measured using a laser micrometer (Z-Mike; Beta Laser Mike, Dayton, OH). The specimens were examined for any structural defects (e.g., nicks, tears, etc.) that may confound the mechanical testing results. The tendons were affixed in a MTS 858 Servohydraulic Tester (MTS Corp., Eden Prairie, MN) with the tendon fibers aligned parallel to the axis of extension. Slightly modified testing procedures were utilized for GT and BTB as described below.

For GT, the specimens were affixed to the MTS using cryogenic grips (Enduratec, Eden Prairie, MN; Figure 1), and were pre-conditioned for 10 cycles (0.5-2.5% strain). A pre-load of 30 N was then applied followed immediately by a ramp to failure (rate = 10% gage length/sec).

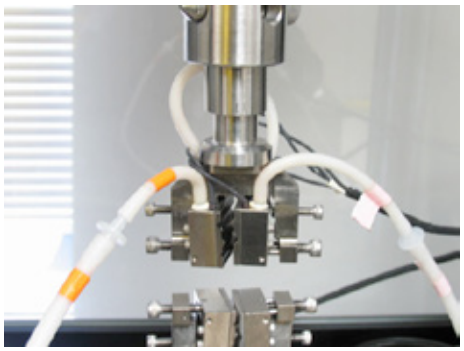

Figure 1: Cryogenic grips used for gripping gracilis tendons.

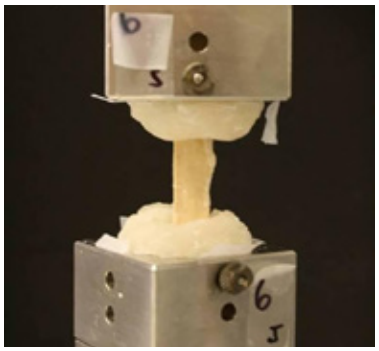

Figure 2: Representative BTB specimen potted in PMMA and affixed in the MTS for testing.

For BTB's, the bone ends of the BTB grafts were potted in polymethylmethacrylate (PMMA) bone cement for fixation to the MTS (Figure 2). A 5N pre-load was applied and the BTB's were cycled through 100 cycles (min load = 50N, max. load = 150N @ 0.5 Hz) to measure cyclic creep strain (defined as the measured grip-to-grip strain at the peak of the 1st and 100th cycle). A 5N pre-load was then again applied followed immediately by a ramp to failure (rate = 10% gage length/sec).

All specimens were kept moist with saline-soaked gauze during testing. Material and structural properties were determined from the load-extension curves and were analyzed using a student's t-test (significance level of $p < 0.05$). All data is reported as average ± standard deviation.

RESULTS

Delivered Dose: Dosimeter reports were used to determine the actual delivered dose which the Pre-Treatment groups received. The Actual delivered doses for the Pre-Treatment groups are shown in Table 1. These dose ranges are typical of the delivered doses normally observed during the Pre-Treatment process.

Group	Target Dose (kGy)	Actual Dose (kGy)
GT	15	13.9 ±1.7
BTB	15	14.9 ±1.6

Table 1: Target and delivered doses for tendons in the Pre-Treatment groups. (Tendons in the control group received no radiation.)

Upon visual examination, tendons showed no noticeable differences between the Control and Pre-Treatment groups (Figure 3).

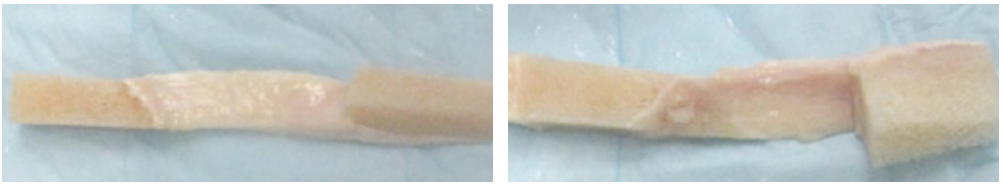

Figure 3: There is no visual difference in the appearance of BTB's in the Control (Left Picture) and Pre-Treatment (Right Picture).

GT: One donor pair was excluded due to machine failure resulting in a final paired sample set of N=9. No significant difference in XSA was observed between the Control and Pre-Treatment groups, 5.39 ±1.37 mm^2 and 4.80 ± 1.35 mm^2, respectively.

During tensile testing, all failures occurred in the tendon mid-substance. There was no difference in either the structural (Figure 4) or material properties (Figure 5) between the Control and Pre-Treatment groups.

BTB: No significant difference in XSA was observed between the Control and Pre-Treatment groups, 80.1 ±14.4 mm^2 and 86.4 ± 19.1 mm^2, respectively.

There was no difference in either the structural (Figure 6) or material properties (Figure 7) between the Control and Pre-Treatment groups. There was also no difference in creep or strain at peak load (Figure 8). Energy to failure was similar for both groups (7,704 ±3,230 N-mm and 6,410 ± 2,241 N-mm, respectively), with no significant differences between Pre-Treatment and Control.

During tensile testing, three specimens failed at the insertion and seven specimens failed in the tendon mid-substance for the Control group and four specimens failed at the insertion and six specimens failed in the tendon mid-substance for the Pre-treatment group.

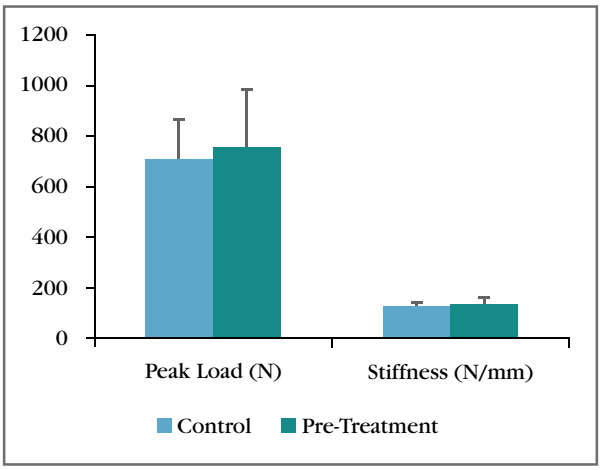

Figure 4: Structural properties (peak load and stiffness) for Gracilis tendons in the Control and Pre-Treatment groups. No significant differences were observed (N=9).

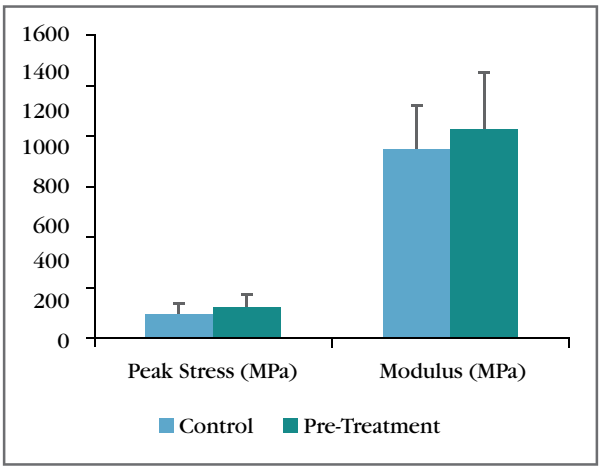

Figure 5: Material properties (peak stress and modulus) for Gracilis tendons in the Control and Pre-Treatment groups. No significant differences were observed (N=9).

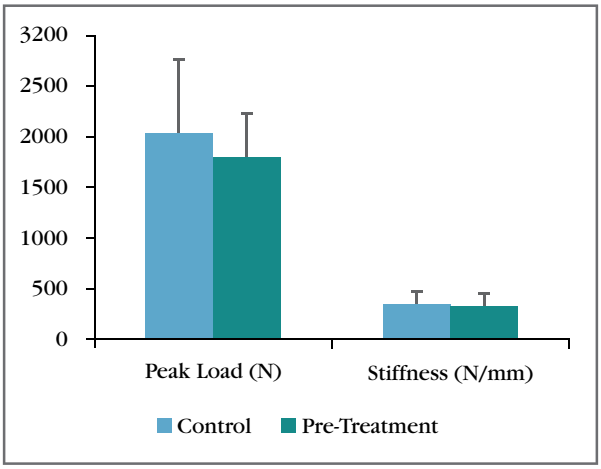

Figure 6: Structural properties (peak load and stiffness) for BTB's in the Control and Pre-Treatment groups. No significant differences were observed (N=10).

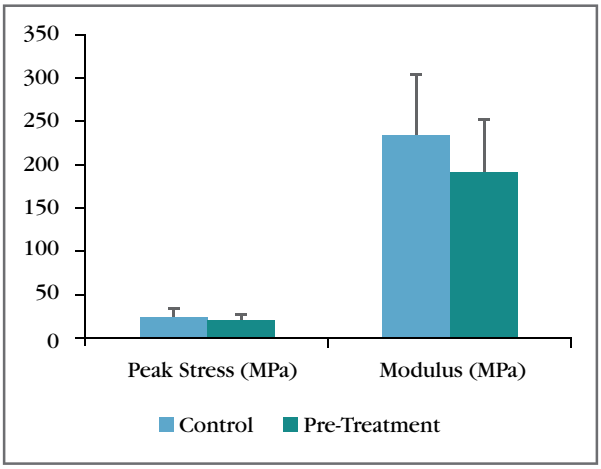

Figure 7: Material properties (peak stress and modulus) for BTB's in the Control and Pre-Treatment groups. No significant differences were observed (N=10).

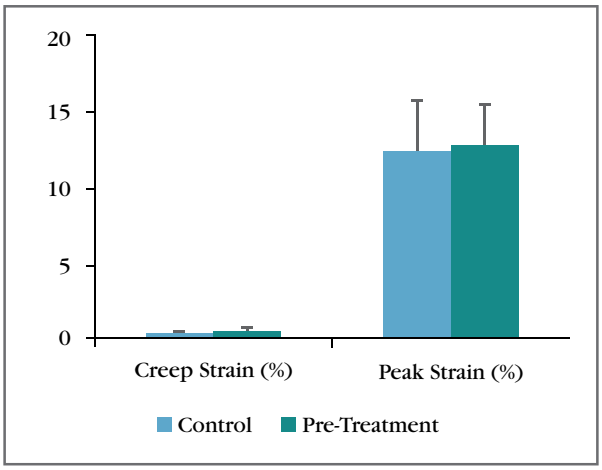

Figure 8: Creep and Strain at Peak for BTB's in the Control and Pre-Treatment groups. No significant differences were observed (N=10).

DISCUSSION

Gamma radiation is routinely used as a means to terminally sterilize tendon allografts, but terminal gamma radiation of allograft tendons can adversely impact mechanical properties in a dose dependent manner.[1,3] Since MTF does not terminally sterilize soft tissue allografts, an alternative procedure to terminal radiation exposure is utilized whereby allograft tendons are qualitatively screened for bioburden prior to processing. Only those allografts where there is either 1) no quantifiable bioburden or 2) the bioburden is susceptible to gamma radiation are then aseptically processed. These later allograft tendons (with susceptible bioburden present) are then exposed to a low-dose Pre-Treatment of gamma radiation prior to processing (i.e., while the allograft is still anatomically attached to surrounding tissue) which may moderate the potential for radiation damage to the allograft tissue. This allows MTF to process tendons in an aseptic manner (utilizing standard cleaning and disinfection procedures) with the ability to maintain sterility throughout the processing steps and avoid the use of harsh terminal sterilization with gamma or electron-beam radiation (or other harmful methods).

The purpose of this study was to demonstrate that Pre-Treatment with low-dose gamma radiation (15 kGy) does not affect the mechanical properties of human allograft tendons. The hypothesis is supported by demonstrating that Pre-Treatment of allograft tendons with low-dose (15 kGy target) gamma radiation has no measurable effect on baseline mechanical properties of GT and BTB.

Although this is an *in vitro* baseline study, the lack of any impact on mechanical properties due to pre-treatment with low-dose radiation exposure prior to processing is supported by the clinical success (with minimal complications) of tendons utilized for ACLR which had been exposed to Pre-Treatment gamma radiation prior to processing.[4] Additionally, it is important to compare the strength of the allograft to the strength of the tissue being replaced. The ACL has a peak load of 1730 N, with a stiffness ranging from 125-190 N/mm when tested ex vivo.[5] In situ loads of the ACL are approximately 110N.[6] Given that the GT can be quadrupled when used for ACLR, the average peak loads for the both GT and BTB following Pre-Treatment are sufficient for use in ACLR.

CONCLUSION

Pre-Treatment of allograft tendons with low-dose (15 kGy) gamma radiation has no measurable effect on mechanical properties.

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