

Soft-Tissue Allografts Terminally Sterilized with an Electron Beam Are Biomechanically Equivalent to Aseptic, Nonsterilized Tendons

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Background: Allograft safety is contingent on effective sterilization. However, current sterilization methods have been associated with decreased biomechanical strength and higher failure rates of soft-tissue allografts. In this study, electron beam (e-beam) sterilization was explored as an alternative sterilization method to preserve biomechanical integrity. We hypothesized that e-beam sterilization would not significantly alter the biomechanical properties of tendon allograft compared with aseptic, nonsterilized controls and gamma-irradiated grafts.

Methods: Separate sets of forty fresh-frozen tibialis tendon allografts (four from each of ten donors) and forty bisected bone-patellar tendon-bone (BTB) allografts (four from each of ten donors) were randomly assigned to four study groups. One group received a 17.1 to 21.0-kGy gamma radiation dose; two other groups were sterilized with an e-beam at either a high (17.1 to 21.0-kGy) or low (9.2 to 12.2-kGy) dose. A fourth group served as nonsterilized controls. Each graft was cyclically loaded to 200 N of tension for 2000 cycles at a frequency of 2 Hz, allowed to relax for five minutes, and then tested in tension until failure at a 100%/sec strain rate. One-way analysis of variance testing was used to identify significant differences.

Results: Tibialis tendons sterilized with both e-beam treatments and with gamma irradiation exhibited values for cyclic tendon elongation, maximum load, maximum displacement, stiffness, maximum stress, maximum strain, and elastic modulus that were not significantly different from those of nonsterilized controls. BTB allografts sterilized with the high e-beam dose and with gamma irradiation were not significantly different in cyclic tendon elongation, maximum load, maximum displacement, stiffness, maximum stress, maximum strain, and elastic modulus from nonsterilized controls. BTB allografts sterilized with the e-beam at the lower dose were significantly less stiff than nonsterilized controls ($p = 0.014$) but did not differ from controls in any other properties. The difference in stiffness likely resulted from variations in tendon size rather than the treatments, as the elastic moduli of the groups were similar.

Conclusions: The biomechanical properties of tibialis and BTB allografts sterilized with use of an e-beam at a dose range of 17.1 to 21.0 kGy were not different from those of aseptic, nonsterilized controls or gamma-irradiated allografts.

Clinical Relevance: E-beam sterilization can be a viable method to produce safe and biomechanically uncompromised soft-tissue allografts.

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A commentary by Samuel A. Taylor, MD, and Robert Marx, MD, MSc, FRCS, is linked to the online version of this article at jbjs.org.

The use of musculoskeletal allografts has proliferated over the past decade, with an estimated 1.5 million allografts distributed annually in the United States¹⁻³. Soft-tissue allografts have been particularly successful in sports medicine applications, with favorable results in common procedures such as ACL (anterior cruciate ligament) reconstruction^{2,4-7}. Allografts can avoid disadvantages such as harvest site morbidity, muscle weakness, altered cosmesis, and limited availability that are often present with autograft use.

However, some recent clinical studies have shown higher failure rates in ACL reconstructions utilizing allografts, especially in younger patients⁸⁻¹⁰. Although the reasons for these increased failures are not well understood, bioburden reduction and terminal sterilization methods may be a factor. Traditionally, the two most common methods of sterilization have been chemical sterilization with ethylene oxide and ionizing radiation in the form of gamma rays⁵. Although ethylene oxide has proven efficacious for reducing the bioburden^{11,12}, its penetration into the tissue is limited and gas residuals have been linked to reduction in tissue remodeling and activation of immunological responses that can lead to graft dissolution¹³⁻¹⁵. Gamma irradiation has become the most prevalent method used by allograft manufacturers for terminal sterilization^{3,16}. Similar to ethylene oxide, gamma radiation is extremely efficient in deactivating microorganisms; however, the biomechanical integrity of the tissue may be compromised with this method, and gamma irradiation has been linked to higher allograft failure rates¹⁷⁻¹⁹.

It has generally been accepted that the loss of biomechanical integrity in musculoskeletal tissue due to gamma irradiation is dose-dependent, with greater detrimental effects occurring at higher doses^{16,20-24}. However, the effect of a low dose of gamma radiation, loosely defined as approximately 15 to 20 kGy, on soft-tissue allografts remains debatable. Balsly et al. reported that a low gamma radiation dose of 18.3 to 21.8 kGy did not significantly reduce the tensile strength or elastic modulus of bone-patellar tendon-bone (BTB) and semitendinosus tendon allografts²⁰, whereas Curran et al. found that BTB allografts exposed to 20 kGy of gamma radiation exhibited a 27% increase in elongation and a 20% decrease in tensile strength compared with nonirradiated controls¹⁶. Other work has shown a decrease of 15% in the tensile strength of BTB allografts subjected to 20 kGy of gamma radiation as well as significant reductions in maximum force, strain energy, and elastic modulus²³.

Alternative methods of sterilization have been explored in an effort to circumvent the potential adverse effects of gamma radiation on the biomechanical properties of soft tissue. Electron beam (e-beam) processing, a nonproprietary technology approved by the U.S. FDA (Food and Drug Administration), is widely used for sterilizing medical products and packaging materials for foods as well as for disinfection of unprocessed bulk crops. In this process, an accelerated beam of electrons kills bacteria by directly breaking DNA chains and creating highly reactive compounds or atoms that induce further chemical destruction²⁵. E-beam sterilization requires only minutes of exposure for effectiveness, compared with hours necessary for gamma irradiation; thus, the degradation of tissue due to heat and free radicals can be appreciably reduced²⁶. Early concerns regarding the limited

penetrability of dense materials by electrons have been addressed through process improvements, including increasing the power of the electron beam to 10 MeV and incorporating two-sided dose delivery²⁷. Several medical device companies have successfully adopted e-beam technology to terminally sterilize their tissue-based products because of the benefits of reduced tissue degradation, well-controlled dose ranges, and rapid turnaround^{25,28}. Encouraged by this progress, our organization has validated that e-beam sterilization of soft-tissue allografts at delivered doses between 9.2 and 21.0 kGy achieves a sterility assurance level (SAL) of 10^{-6} , as measured in accordance with the ANSI/AAMI/ISO (American National Standards Institute/Association for the Advancement of Medical Instrumentation/International Organization for Standardization) 11137 standards.

Unlike the extensive research available on the biomechanical impact of gamma radiation^{16,20-24}, few studies have investigated how e-beam sterilization influences soft-tissue biomechanics^{29,30}. Furthermore, those e-beam studies used high radiation doses (up to 50 kGy) and incorporated additives for tissue protection at such doses (free radical scavengers or CO₂) that are atypical of the allograft sterilization process employed by our organization. Accordingly, the purpose of this study was to elucidate the effects of e-beam sterilization at two doses (17.1 to 21.0 and 9.2 to 12.2 kGy) on the biomechanical behavior of tibialis tendon and BTB allografts as well as to compare the results with those of otherwise similar gamma-irradiated and nonsterilized tendons. Our hypothesis was that e-beam sterilization would not significantly alter the structural and material properties of soft-tissue allografts compared with gamma-irradiated tendon allografts and nonsterile controls.

Materials and Methods

Specimen Preparation

Paired BTB allografts from ten donors (six male and four female; mean age, 57.8 years; age range, forty-nine to seventy-two years) and paired anterior and posterior tibialis tendons from a different group of ten donors (five male and five female; mean age, 65.1 years; age range, forty to seventy-four years) were aseptically recovered and processed as fresh-frozen grafts in accordance with current soft-tissue standard manufacturing procedures that included a commonly used proprietary bioburden reduction treatment (Allowash; LifeNet Health, Virginia Beach, Virginia). Previous results in our laboratory indicated that there were no significant biomechanical differences between left and right or anterior and posterior tibialis tendons (see Appendix). BTB allografts were bisected and trimmed to a width of ≤ 12 mm to yield four grafts per donor. Therefore, a total of forty BTB and forty tibialis tendon allografts were used in the study. All donors had consented to research use.

The tibialis tendon and BTB allografts were separated into two groups by tendon type. The tendons within each of these groups were then randomly assigned to one of four study groups ($n = 10$ per group): aseptically processed, nonsterilized (nonsterile group); e-beam sterilized at a 17.1 to 21.0-kGy dose (e-beam high group); e-beam sterilized at a 9.2 to 12.2-kGy dose (e-beam low group); and gamma-irradiated at a 17.1 to 21.0-kGy dose (gamma group). The nonsterilized tendons served as the untreated control group, and the e-beam and gamma-sterilized tendons were the treatment groups. The gamma and e-beam radiation doses were selected on the basis of dose validation studies currently established for routine production at our facility in accordance with ISO Standard 11137-2 Method 2B³¹. Process protocols were established with the service provider (Synergy Health Americas; San Diego, California) prior to e-beam sterilization to achieve a uniform dose delivery to the tissues. On the basis of the recommendation by the service provider, protective agents were deemed unnecessary because they are typically only used at doses of >30 kGy.

TABLE I Structural Properties of Tibialis and BTB Allografts Tested to Failure*

Study Group	Tibialis			BTB		
	Max. Displacement (mm)	Max. Load (N)	Stiffness (N/mm)	Max. Displacement (mm)	Max. Load (N)	Stiffness (N/mm)
Nonsterile	10.10 ± 6.38	606.73 ± 283.52	176.69 ± 62.92	8.15 ± 2.73	1494.68 ± 497.78	302.93 ± 31.88
E-beam low	9.29 ± 3.68	876.38 ± 310.73	177.09 ± 46.16	8.74 ± 2.33	1144.16 ± 508.09	189.85 ± 69.47
E-beam high	8.96 ± 1.86	660.24 ± 312.12	149.90 ± 51.33	7.17 ± 2.61	1215.75 ± 659.83	231.28 ± 60.23
Gamma	8.09 ± 1.84	597.09 ± 280.32	147.21 ± 49.18	8.45 ± 1.40	1218.37 ± 563.86	242.90 ± 102.02
P value†	0.878	0.142	0.174	0.468	0.523	0.014‡

*The values are given as the mean and the standard deviation. †One-way ANOVA test indicating difference among the four study groups. ‡Significant difference in stiffness of the BTB samples between the e-beam low and nonsterile study groups according to the Tukey HSD post hoc test.

Specimens treated with gamma irradiation were maintained frozen on dry ice in accordance with standard production procedures.

Biomechanical Testing

All grafts were stored at -70°C until testing, at which time they were thawed at room temperature and then warmed in a water bath set to physiologic temperature (37°C). If necessary, BTB allografts were trimmed to a minimum tendon length-to-width ratio of 3:1 (with tendon length measured as the distance between the bone blocks). The cross-sectional dimensions were measured in the unloaded state with use of a CCD (charge coupled device) optical micrometer (LS-7030; Keyence, Elmwood Park, New Jersey). The tibial bone blocks were then potted with use of a body filler resin (Bondo; 3M, St. Paul, Minnesota) in custom molds. Both ends were secured in pneumatic clamps mounted on a materials testing system (ElectroPuls E3000; Instron, Norwood, Massachusetts).

Similarly, each tibialis specimen was trimmed to achieve a minimum tendon length-to-width ratio of 3:1, and its cross section was measured. A 50 to 60-mm section in the middle of the tendon was designated as the testing area, and the specimen ends on either side of this section were secured in 60-grit sandpaper to prevent slippage in the pneumatic clamps. Tendon length between the sandpaper was measured in an unloaded state with use of calipers. Ink marks were made at the clamp-tendon interface to evaluate possible slippage at the grips.

A dynamic testing procedure previously established for cyclic tendon testing^{30,32,33} was programmed into the testing system software (WaveMatrix; Instron) for use in this study. Each tendon was preconditioned under cyclic loading that ramped from 0 to 20 N at 0.5 Hz for ten cycles. After preconditioning, the grip-to-grip distance taken from the position of the actuator was defined as the initial gauge length. The grafts were then subjected to 2000 cycles of cyclic tensile loading that sinusoidally ramped from 0 to 200 N at a rate of 2 Hz. Following the cyclic sub-failure testing, the tissues were unloaded and allowed to relax for five minutes. Lastly, the specimens were loaded in tension to failure at a displacement rate of 100% of the initial gauge length per second. Load was measured with use of a load cell with a $\pm 5\text{-kN}$ capacity and 0.1-N resolution (2527-103 Dynacell; Instron), and grip-to-grip displacement was measured through the actuator (resolution, 0.001 mm). All testing was conducted in a custom environmental chamber that maintained physiologic temperature ($37^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and constantly misted the specimens with phosphate buffered saline solution to provide tissue hydration throughout testing.

Biomechanical Property Calculations

Cyclic tendon elongation was expressed as a percentage and defined as the strain difference following the completion of the 2000 cycles of sub-failure loading: $([L_{2000} - L_0]/L_0) \times 100$, where L is the recorded actuator displacement at the indicated cycle and L_0 is the initial grip-to-grip distance. Structural and material properties of each specimen were calculated from load-displacement data and

stress-strain data, respectively, in the testing to failure. Mean strain was determined as the ratio of actuator displacement to the initial specimen gauge length. Initial tendon measurements were used to convert the structural properties to material properties. Stiffness and elastic modulus were calculated as the slope of the linear portion of the load-displacement and stress-strain curves, respectively, for the failure portion of the test. For all tests, the points used in the slope calculations were selected at 20% and 80% of the absolute peak in the load-displacement or strain-stress curve.

Statistical Analyses

The mean and standard deviation of the cyclic tendon elongation, stiffness, elastic modulus, maximum load and displacement, and maximum stress and strain were calculated for the tibialis tendon and BTB allografts from each study group. Statistical software (SigmaPlot version 12.3; Systat Software, San Jose, California) was used to perform one-way ANOVA (analysis of variance) to identify significant differences in structural and material properties among the study groups within each tendon type. The study group was treated as the factor, and cyclic tendon elongation, stiffness, elastic modulus, maximum displacement, maximum strain, maximum load, and maximum stress were treated as the response variables. All properties were analyzed for normality with use of the Shapiro-Wilk test. If it was determined that the data were not normally distributed, a Kruskal-Wallis one-way ANOVA on ranks was performed. The Tukey HSD (honestly significant difference) post hoc test was used for comparisons of the group means if a significant difference was found. All reported p values are two-tailed, and a p value of 0.05 was considered significant. Statistical analyses of the four tibialis tendon allograft study groups were conducted separately from those of the BTB allograft study groups, as a direct comparison of the two graft types was not an aim of this study.

Source of Funding

No external funds were utilized for this study. E-beam sterilization services were performed at no cost by Synergy Health, which had no influence over the design, analysis, or interpretation of the study data.

Results

The initial gauge length and cross-sectional area of the tibialis tendon allografts were 52.89 ± 4.33 mm and 48.86 ± 11.67 mm², respectively, and those of the BTB allografts were 34.50 ± 4.91 mm and 60.73 ± 12.54 mm².

Four tibialis tendon allografts (one from each study group) and three BTB tendon allografts (one each from the gamma, e-beam high, and e-beam low study groups) failed during the cyclic portion of the testing. These tendons were included in the failure analyses but were excluded from the cyclic tendon elongation

TABLE II Material Properties of Tibialis and BTB Allografts*

Study Group	Tibialis				BTB			
	Max. Stress (MPa)	Max. Strain (mm/mm)	Elastic Modulus (MPa)	Cyclic Elongation (%)	Max. Stress (MPa)	Max. Strain (mm/mm)	Elastic Modulus (MPa)	Cyclic Elongation (%)
Nonsterile	13.52 ± 5.97	0.19 ± 0.13	213.13 ± 98.86	7.99 ± 5.10	25.79 ± 9.02	0.22 ± 0.06	186.08 ± 42.00	5.91 ± 1.18
E-beam low	19.07 ± 6.34	0.17 ± 0.07	206.71 ± 88.87	4.92 ± 2.05	18.87 ± 8.87	0.26 ± 0.08	122.38 ± 53.02	6.91 ± 4.47
E-beam high	12.71 ± 6.57	0.17 ± 0.04	152.64 ± 75.10	5.92 ± 3.16	18.87 ± 11.50	0.21 ± 0.07	128.21 ± 46.25	4.60 ± 2.60
Gamma	13.37 ± 5.85	0.15 ± 0.11	179.02 ± 73.16	6.08 ± 2.66	21.87 ± 11.33	0.25 ± 0.04	146.80 ± 70.44	4.60 ± 3.05
P value†	0.096	0.810	0.372	0.228	0.393	0.275	0.052	0.057

*The values are given as the mean and the standard deviation. †One-way ANOVA test indicating differences across the four study groups.

calculations because they ruptured before completing the full 2000 cycles. Considerations possibly bearing on the reasons for these specific atypical failures are discussed in the Appendix.

For tibialis tendon allografts sterilized with both e-beam doses and with gamma radiation, the calculated structural properties of maximum load, maximum displacement, and stiffness were not significantly different from those for the nonsterilized controls (Table I). Likewise, the material properties of maximum stress, maximum strain, elastic modulus, and cyclic tendon elongation for all three sterilized groups were not significantly different from those for the nonsterilized controls (Table II).

For BTB allografts, the structural properties of maximum load and maximum displacement and the material properties of maximum stress, maximum strain, elastic modulus, and cyclic tendon elongation did not differ significantly among the study groups (Tables I and II). BTB allografts sterilized with the e-beam at the lower dose were significantly less stiff than nonsterilized controls ($p = 0.014$), but they had elastic modulus values similar to those of the other three study groups.

Discussion

The annual rise in ACL reconstructions and the increasing use of allografts in such repairs have driven the need for an effective sterilization method that preserves the biomechanical integrity of the allografts. The results of this study indicate, with one exception, that e-beam irradiation at doses of 9.2 to 12.2 and 17.1 to 21.0 kGy and gamma irradiation at doses of 17.1 to 21.0 kGy did not significantly alter the biomechanical properties of tibialis tendon and BTB allografts compared with aseptically processed, nonsterilized control allografts.

Maximum loads for tibialis tendons in the e-beam high (660.24 ± 312.12 N), e-beam low (876.38 ± 310.73 N), gamma (597.09 ± 280.32 N), and nonsterilized groups (606.73 ± 283.52 N) were comparable with maximum loads reported in the literature for nonirradiated anterior (777 ± 174 N) and posterior (889 ± 259 N) tibialis tendons³⁴. Additionally, the similar elongation behavior observed among all treatments during cyclic loading was consistent with the work of Seto et al., which showed that tendon (viscoelastic) properties were minimally affected by e-beam irradiation even at a high dose of 50 kGy²⁹.

Similarly, previously reported values for failure load (1139 to 2570 N) and failure strength (23 to 32 MPa) of native BTB tendons correspond well with the maximum load and maximum stress results in the present study (1144 to 1495 N and 19 to 26 MPa, respectively)^{20,34}. In addition, the present results are consistent with those in the study by Hoburg et al., in which BTB allografts sterilized with an e-beam at doses of 15, 25, and 34 kGy had mechanical behavior indistinguishable from that of nonsterile controls³⁰. The failure loads measured after e-beam treatment were slightly higher in the study by Hoburg et al. than in the present study, possibly because of the use of CO₂ to avoid undesired reactions in the tissue during irradiation. The cyclic tendon elongation values of $4.60\% \pm 2.60\%$ (e-beam high) and $6.91\% \pm 4.47\%$ (e-beam low) that we measured were higher than those reported by Hoburg et al. ($3.28\% \pm 0.96\%$ for 15 kGy and $4.02\% \pm 1.12\%$ for 25 kGy), but this could be due to the tenfold greater number of sub-failure cycles in our study or to differences between the measurement methods in the studies.

We detected a significant difference in stiffness between the e-beam low and control groups of BTB allografts. However, stiffness is a structural property that does not take into account variations in specimen dimensions. Once tissue dimensions were used to calculate the elastic modulus (an intrinsic material property), no difference was present.

Our finding that gamma-irradiated tendons were also not biomechanically different from nonsterilized tendons was not surprising given the recent literature. Although early studies on sterilization effects showed that high gamma radiation doses reduced the biomechanical properties of various musculoskeletal tissue, more recent work evaluating gamma sterilization at lower doses showed no significant differences compared with unsterilized tendons^{20,35}.

The present study focused on e-beam and gamma radiation dose ranges of <21 kGy; although such doses are commonly used in the allograft industry, they are lower than the sterilization doses that have typically been examined in the literature, and for which biomechanical decrements^{23,30,36} and/or adverse remodeling effects^{37,38} have been documented. To avoid such negative effects, most tissue banks have opted to use irradiation with doses no higher than 25 kGy. When combined with improved donor screening and selection processes, meticulous aseptic techniques

for tissue recovery and processing, and robust bioburden reduction treatments, such sterilization methods have been validated to achieve an SAL of 10^{-6} , meaning that the probability that a particular graft remains nonsterile is only one in one million. Other tissue banks have reported data on validated processes that achieve these SAL levels with use of a minimum sterilization dose of 9.2 kGy^{39} , supporting our dose selection for this study.

Importantly, our study addressed the effects of e-beam and gamma sterilization on soft-tissue allograft biomechanics only prior to implantation. The *in vivo* response of allografts treated with these terminal sterilization protocols remains unknown. The tensile testing protocol used was, however, consistent with a number of *in vivo* conditions, including temperature, hydration, and cyclic loading to quasiphysiologic strain levels.

All tissues used in this study were treated with use of a patented process (Allowash) to reduce the bioburden. Although proprietary validation data and associated literature provided by LifeNet Health indicate that Allowash does not significantly affect soft-tissue properties, caution must be used in extrapolating our data to tissues not treated with this process. However, as the majority of tissue banks utilize Allowash or a very similar cleaning and disinfecting process, the data from this study should be widely applicable.

One concern regarding irradiation of soft tissue is possible fragmentation of collagen and reduction of collagen crosslink density. Observations of such effects are characteristic of studies in which tissue is subjected to high radiation doses (typically $\geq 25 \text{ kGy}$)^{22,29,40}. The use of protective agents such as crosslinking agents and free radical scavengers aims to offset such effects. Most research proposing the use of protective agents is concerned with radiation doses of $>25 \text{ kGy}^{41-43}$, which are higher than the doses of interest in our study or those used in our standard tissue sterilization procedures. As we were evaluating doses of $<25 \text{ kGy}$, the perceived benefit of protective agents was questionable and their use might have been a confounding variable.

In summary, this study showed that soft-tissue allografts terminally sterilized with an e-beam at a dose between 9 and 21 kGy or with "low-dose" gamma radiation had biomechanical behavior not significantly different from that of similar nonsterilized tendons. Because of a negative perception that gamma irradiation weakens soft tissue, surgeons are using aseptic allografts to safeguard biomechanical integrity, but this comes at the risk of implanting a nonsterile graft. The present results support the conclusion that tissues sterilized with either an e-beam or gamma radiation at the specified doses (which can provide an SAL of 10^{-6}) maintain biomechanical properties similar to those of nonirradiated tendon allografts.

Appendix

(eA) A description and tables pertaining to the comparisons among tendon sources within the two types of tendons and to the specimens that failed during cyclic testing are available with the online version of this article as a data supplement at jbjs.org. ■

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