

Gamma Irradiation: Effects on Biomechanical Properties of Human Bone-Patellar Tendon-Bone Allografts

Bradley M. Fideler, MD, C. Thomas Vangsness, Jr.,* MD, Bin Lu, MS, Carlo Orlando, MD, and Tilman Moore, MD

From the Department of Orthopaedic Surgery, University of Southern California School of Medicine, Los Angeles, California

ABSTRACT

Sixty 10-mm bone-patellar tendon-bone allografts from young human donors were placed into four test groups, a control fresh-frozen group and three fresh-frozen irradiated groups. The irradiated groups were exposed to 2.0, 3.0, or 4.0 Mrad of gamma irradiation. The specimens were tested to tensile failure. The initial biomechanical strength of fresh-frozen allografts was reduced up to 15% when compared with fresh-frozen controls after 2.0 Mrad of irradiation. Maximum force, strain energy, modulus, and maximum stress demonstrated a statistically significant reduction after 2.0 Mrad of irradiation ($P < 0.01$). Stiffness, elongation, and strain were reduced but not with statistical significance. A 10% to 24% and 19% to 46% reduction in all biomechanical properties were found after 3.0 ($P < 0.005$) and 4.0 ($P < 0.0005$) Mrad of irradiation, respectively. After irradiation with a 4.0 Mrad dose, the ultimate load was below that of reported values for the human anterior cruciate ligament. It is clinically important to observe and document changes in human ligaments that result from currently used doses of gamma irradiation. The results from this study provide important information regarding the initial biomechanical properties of fresh-frozen human bone-patellar tendon-bone allografts after bacterial sterilization with gamma irradiation. The current accepted dose for sterilization is between 1.5 and 2.5 Mrad. There appeared to be a dose-dependent effect of irradiation on all the biomechanical parameters studied. Four of seven parameters were found to be reduced after 2.0 Mrad of irradiation. Reductions were found in all parameters after 3.0 and 4.0 Mrad of irradiation.

The use of bone and soft tissue allografts for knee ligament reconstruction is of interest to orthopaedic surgeons.^{11,16,17,18,29,30} Allografts offer several advantages over autografts, including decrease in donor site and overall surgical morbidity, preservation of normal tissue, reduced operative time, unaltered patellofemoral tracking, and improved cosmesis. They are also a good alternative for patients who have failed a previous autogenous patellar tendon reconstruction.¹⁸ Several investigators have compared allografts with autografts and found few differences. An animal study has shown allografts to have a slower rate of biologic incorporation,^{16,17} but frozen and freeze-dried allografts are similar to autografts histologically and biomechanically 8 to 12 months after ACL replacement.^{2,5,10,19,23,24,30,32,33,36}

Although the incidence of serious disease transmission with allografts is very low, there is concern regarding the sterility and safety of bone and soft tissue allografts. Tissue banks obtain allografts by careful donor screening and clean or aseptic procurement. The allografts are then preserved by either freezing or freeze drying. Surgeons and tissue bank personnel are concerned that allografts processed by these methods may harbor viruses that can transmit disease such as human immunodeficiency virus (HIV)⁸; therefore, allografts have been sterilized with either ethylene oxide or gamma irradiation. Ethylene oxide processing has been associated with intraarticular reaction requiring removal of the graft.^{18,20,29} Gamma irradiation may be preferable because it is presumed not to cause serious inflammatory reactions, and because gamma irradiation is an effective bacterial sterilizer. It is reported that HIV resembles other retro-viruses in its sensitivity to gamma irradiation.²³ Doses of 2.5 Mrad of gamma irradiation have been shown to inactivate HIV in liquid plasma and culture medium, but higher dosages were required in frozen plasma.^{21,31} These results may not be directly transferable to bone and soft tissue, particularly in the dry and frozen state. Preliminary indications showed that this dosage may be inadequate, and higher doses may be required.^{9,12}

*Address correspondence and reprint requests to C. Thomas Vangsness, MD, Department of Orthopaedic Surgery, University of Southern California School of Medicine, 1510 San Pablo Street, Suite 322, Los Angeles, CA 90033.

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Sterilization with gamma irradiation alters the initial strength and mechanical properties of the graft. Irradiation of goat bone-patellar tendon-bone allografts showed significant reductions after a 3.0 Mrad dose in five of eight parameters, but none after a 2.0 Mrad dose.^{13,14} The effects of this processing technique on the biomechanical properties of human fresh-frozen bone and soft tissue allografts is largely unknown. The purpose of this study was to examine the biomechanical properties of human fresh-frozen bone-patellar tendon-bone allografts after clinically relevant incremental doses of gamma irradiation.

MATERIALS AND METHODS

Fifteen young cadaveric donors, who died from sudden or traumatic death, were selected for study. Donors with evidence of an injury to the patella or patellar tendon or pre-existing illnesses, such as HIV and hepatitis, were excluded.

Thirty bone-patellar tendon-bone specimens were harvested within 72 hours of death, wrapped in sterile gauze soaked in normal saline, sealed in individual plastic bags, and frozen to -70°C until processing. Specimens were divided longitudinally, permitting 60 one-half bone-patellar tendon-bone allograft specimens to be divided into 4 groups. Each specimen was marked to designate the harvest location. The four study groups were 1) no irradiation, 2) irradiated with a 2.0 Mrad dose, 3) irradiated with a 3.0 Mrad dose, and 4) irradiated with a 4.0 Mrad dose. The test groups were packed in dry ice in specially designed coolers for transfer to an irradiation facility (J. L. Sheppard and Associates, Inc., San Fernando, California). The specimens were kept frozen at all times and dry ice was added as needed during the irradiation to avoid thawing from associated heat. The specimens were irradiated using a 60 Cobalt source at a strength of 10,000 Ci and 1.5 Mrad an hour. Dosimetry to within 5% error was calculated (dose rate \times exposure time) and verified with dosimeters. After irradiation, the specimens were returned to the freezer where they remained at -70°C until biomechanical testing (range of days, 14 to 90; average, 45).

The allografts were thawed in normal saline at room temperature on the day of testing. All specimens were trimmed to a 10 mm width; length was determined with digital calipers accurate to 0.05 mm from the average of four bone-to-bone measurements made on the posterior aspect of the tendon. Width and thickness measurements were made at four equally spaced locations along the length of the tendon. According to established techniques,^{22,34} the cross-sectional area was computed assuming a rectangular shape from the average of four measurements.

Seven biomechanical parameters were selected to evaluate the effects of gamma irradiation on human bone-patellar tendon-bone allografts. These parameters have been well described regarding tensile testing of tissues.^{4-6,13,14,26,28,35} Four structural properties (stiffness, maximum force, elongation to maximum force, and strain energy to maximum force) and three material properties (tendon modulus, maximum stress, and strain to maximum stress) were measured for each allograft specimen.

A 1/8-inch Steinmann pin was inserted through the tibial bone plug to anchor the graft, and the specimens were potted in auto body filler (Figs. 1 and 2). Care was taken to avoid contact with the soft tissue or insertion sites. The potted specimens were placed into specially designed grips for tensile testing. The specimens were mounted onto the materials testing machine and preloaded to 5 N (Fig. 3). Specimens were then preconditioned by cyclic testing, using a constant deformation (1% strain) for 10 cycles and tested to failure in tension at a constant strain rate of 100% of initial length per second. Grip-to-grip displacement was used to determine the length changes in each allograft specimen tested. Surface strain of the tendon midsubstance was measured optically between the outer two stain lines using a high-speed video camera that interfaced with a video-dimension analyzer. All testing was recorded on a MTS (MTS Systems Corp., Minneapolis, Minnesota) chart recorder and computer for storage and calculations. The mechanism of tendon failure was documented after each test. The Student's *t*-test was used to compare the biomechanical parameters studied for each level of irradiation with those for the fresh-frozen control.

RESULTS

There were no statistically significant differences in the preirradiation tissue dimensions between the irradiated groups and the fresh-frozen controls ($P > 0.05$). There were no significant tissue dimensions differences ($P > 0.05$) with graft location. Irradiation caused no statistically significant ($P > 0.05$) changes in graft dimensions.

The structural properties of maximum load, stiffness, maximum elongation, and strain energy for control and irradiated specimens are presented in Table 1. There was a trend showing dose-dependent effects of all four properties. Statistically significant differences were found in maximum force and strain energy after 2.0 Mrad of irradiation (Table 1). Stiffness and elongation were reduced after 2.0 Mrad but not significantly (Table 1). All four material structural properties showed a statistically significant reduction after 3.0 and 4.0 Mrad of irradiation (Table 1).

The material properties of tendon modulus, maximum

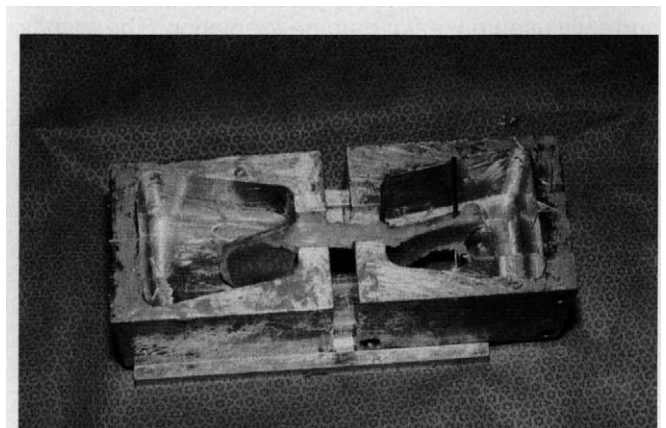


Figure 1. The specimen was placed into specially designed molds for potting.

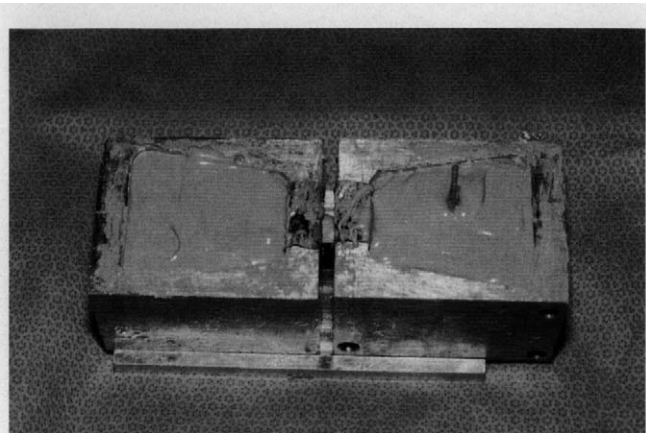


Figure 2. The specimen was potted with auto body filler.

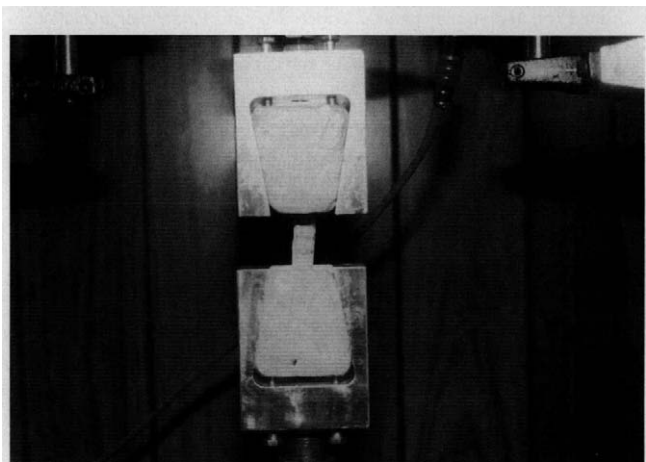


Figure 3. The specimen was mounted on the MTS machine in a specially designed jig.

TABLE 1
Effects of Irradiation on Structural Properties of Allografts

Group	Stiffness (N/mm)	Max force (N)	Max elongation (mm)	Strain energy (N·m)
Frozen control	633	2552	15.73	12.4
2 Mrad	579 ^a	2169 ^b	15.37 ^c	11.3 ^d
3 Mrad	531 ^b	1990 ^c	14.15 ^e	10.5 ^e
4 Mrad	341 ^c	1376 ^c	12.74 ^e	7.1 ^e

^a Not significantly different from the control value, $P = 0.111$.

^b Significantly different from the control value, $P = 0.002$.

^c Not significantly different from the control value, $P = 0.406$.

^d Significantly different from the control value, $P = 0.003$.

^e Significantly different from the control value, $P < 0.0001$.

stress, and maximum strain for the fresh-frozen controls and irradiated groups are presented in Table 2. Statistically significant reductions were found in the modulus and maximum stress after 2.0 Mrad of irradiation (Table 2). Strain also was reduced but not significantly (Table 2). All material properties studied showed a statistically significant reduction after 3.0 and 4.0 Mrad (Table 2).

The analysis of the failure sites is presented in Table 3. Midsubstance failure was most common in the nonirradiated control with 11 of 15 (73%) failing in midsubstance.

TABLE 2
Effects of Irradiation on Material Properties

Group	Modulus (MPa)	Max stress (MPa)	Max strain (%)
Frozen control	812	63.8	13.4
2 Mrad	771 ^a	55.6 ^b	13.2 ^c
3 Mrad	698 ^d	48.5 ^d	12.1 ^b
4 Mrad	454 ^d	35.3 ^d	10.5 ^d

^a Significantly different from the control value, $P = 0.0067$.

^b Significantly different from the control value, $P = 0.005$.

^c Not significantly different from the control value, $P = 0.6131$.

^d Significantly different from the control value, $P < 0.0001$.

TABLE 3
Effects of Irradiation on Failure Site ($N = 15$)

Group	Midsubstance failure	Insertion failure	Combination failure
Frozen control	11	2	2
2 Mrad	11	3	1
3 Mrad	9	4	2
4 Mrad	6	7	2

Failure at the insertion site increased with each dose of irradiation, with 7 of 15 (47%) failing at the insertion site after 4.0 Mrad of irradiation.

DISCUSSION

Musculoskeletal allografts, including bone, cartilage, tendons, and ligaments are used in reconstructive surgery for thousands of orthopaedic patients each year. Although the incidence of transmitting serious disease with allografts is very low, careful donor screening and tissue processing are essential to minimize the risk to the recipient. Guidelines used for processing bone and soft tissue allografts must be based on sound scientific knowledge of safety and strength.¹

When considering the use of a bone-patellar tendon-bone allograft for knee reconstructive surgery, consideration of patient safety is essential. The estimated probability of transmitting the HIV in bone and soft tissue allografts is reported to be as low as one in more than 1 million.³ In 1985, a donor in the "window" stage, who was HIV-seronegative but infected with HIV, transmitted the virus through a bone-patellar tendon-bone allograft to a patient who later converted to HIV-seropositive. Two of three other patients obtaining musculoskeletal allografts from this patient have also become seropositive.¹⁸

To avoid the transmission of infectious bacterial diseases, some tissue banks sterilize allograft tissues with gamma irradiation. Most tissue banks use doses between 1.5 and 2.5 Mrad based on published reports that these doses inactivate HIV.^{13,14} Another study has shown that after exposure to less than 2.5 Mrad of irradiation, HIV was still infectious to human lymphocytes.³¹ The study by Spire et al.³¹ was done with liquid cultures. However, it is believed that 2.5 Mrad of irradiation may be inadequate to inactivate HIV in a frozen bone and soft tissue allograft.⁹ A previous study conducted by Fideler et al.¹² on HIV-infected human fresh-frozen bone-patellar tendon-bone

allografts discovered HIV-positive allografts after irradiation with 2.5 Mrad when tested with the polymerase chain reaction test.

Larger doses of gamma irradiation may be required to inactivate HIV in soft tissue; it then becomes critical to know the biomechanical effects of these doses of gamma irradiation. These data showed dose-dependent effects of gamma irradiation on the biomechanical properties of these allografts. Statistically significant reductions were found in all parameters studied after 3.0 and 4.0 Mrad of irradiation.

The biomechanical properties obtained for the nonirradiated controls in the present study compared favorably with similar data on human bone-patellar tendon-bone grafts used in knee ligament reconstruction.²⁵ The data were consistent with reductions found by Gibbons et al.¹³ in fresh-frozen goat bone-patellar tendon-bone allografts irradiated with 3.0 Mrad. However, our data differ in that reductions were found after 2.0 Mrad of irradiation in four of seven parameters studied; whereas Gibbons et al. found no reductions after 2.0 Mrad. This study was consistent with previous studies on human patellar tendon, although previous biomechanical studies were limited in the number of specimens and statistical data.^{7,15,27}

Our study provided important information concerning the effects of gamma irradiation on the initial biomechanical properties of human fresh-frozen bone-patellar tendon-bone allografts. There are several questions regarding the use of bone-tendon-bone allografts for ACL reconstruction. It has yet to be determined whether the reductions in biomechanical properties after irradiation have a significant effect on the early mobilization and rehabilitation or the final ACL reconstruction outcome. Further studies must be done to determine the effect of preservation and sterilization on the biologic response of allografts over time and on the ability of the patellar tendon allograft to function mechanically and physiologically as a ligament replacement.

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