Mechanical Properties of Patellar Tendon Allografts Subjected to Chemical Sterilization

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Purpose: We tested the hypothesis that the preimplantation mechanical properties of BioCleanse-treated bone–patellar tendon–bone (BPTB) allografts are not significantly different from those of untreated specimens. **Methods:** For this controlled laboratory study, specimens were harvested as central third or hemi-BPTB units from both knees of 17 cadaveric tissue donors (11 men and 6 women) aged 19 to 88 years. Donor-matched specimens (20 per group) were randomly assigned to either BioCleanse-treated or untreated control groups. Specimens were subjected to 25 minutes of pretensioning at 89 N and then cyclically loaded under longitudinal tension between 50 N and 250 N for 1,000 cycles at 1 Hz, followed by ramp to failure at 50 mm/min. **Results:** No statistically significant difference was found between untreated and BioCleanse-treated specimens in stiffness (235.3 ± 37.6 N/mm *v* 222.3 ± 53.4 N/mm, $P = .37$), cyclic creep (0.38 ± 0.42 mm *v* 0.40 ± 0.26 mm, $P = .81$), maximum force (1,685.7 ± 471.6 N *v* 1,807.0 ± 657.8 N, $P = .47$), or ultimate stress (29.0 ± 9.8 MPa *v* 29.0 ± 12.8 MPa, $P = .98$). **Conclusions:** The preimplantation mechanical properties of BPTB allografts treated with BioCleanse are not significantly different from those of untreated controls. **Clinical Relevance:** This laboratory study compares the biomechanical properties of chemically treated allografts, which are currently being used in anterior cruciate ligament reconstruction, with those of nontreated fresh-frozen allografts. **Key Words:** Allograft—Anterior cruciate ligament—Biomechanical—Reconstruction—Sterilization.

Musculoskeletal allografts are gaining acceptance as a valuable option for anterior cruciate ligament (ACL) reconstruction in orthopaedic surgery because studies have shown results comparable to those of autograft tissue reconstruction.[1-7] The advantages of using allografts for ACL reconstruction include unaltered patellofemoral tracking, no donor-site morbidity, decreased overall surgical morbidity, decreased operative time, improved cosmesis with smaller surgical incisions, and decreased overall cost.[5,6] Allografts are particularly useful in patients for whom a previous autogenous patellar tendon reconstruction has failed and in patients with complex multiple ligament reconstruction.[7-9]

A primary disadvantage of allografts is the risk of viral or bacterial disease transmission, as highlighted by the 2001 death of a 23-year-old man from *Clostridium sordellii* sepsis after the transplantation of a contaminated allograft.[10] After this incident, the Centers for Disease Control and Prevention investigated and identified 26 cases of infection associated with musculoskeletal allografts.[11] To minimize the risk of infection associated with allografts, several methods of processing and sterilization have been developed. Donor screening, aseptic processing, and obtaining cultures from the tissues decrease the risk of disease transmission. However, a residual risk of infection remains, as evidenced by hepatitis C virus infection in

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4 patients who received musculoskeletal allografts from a seronegative donor in 2002.[12]

Therefore, terminal sterilization techniques have been developed. Two of the most common methods that have been used are ethylene oxide and gamma irradiation. Although ethylene oxide did not appear to compromise the mechanical properties of the tissue, it was found to cause intra-articular synovial and immune reactions.[13-15] Therefore, gamma irradiation has become widely used as a method of terminal sterilization.[16] It has been shown to be virucidal and bactericidal and has not been reported to induce an inflammatory response.[17,18]

However, gamma-irradiation has been shown to compromise the mechanical and biologic properties of allograft tissue. Studies have shown that bone–patellar tendon–bone (BPTB) allografts sterilized with gamma-irradiation have a dose-dependent reduction in their mechanical properties.[19-23] In one study, maximum force was reduced by 26% in specimens exposed to 4 Mrad of irradiation compared with untreated control specimens.[19] In another study, a dose-dependent reduction in mechanical properties was shown with a 15%, 22%, and 46% decrease in ultimate strength in grafts exposed to irradiation doses of 2, 3, and 4 Mrad, respectively.[20] Additionally, specimens treated with low-dose (2 Mrad) irradiation have been shown to elongate 27% more than untreated grafts when subjected to submaximal cyclic loading.[23]

Therefore, new methods of processing allografts are needed that eliminate viruses, fungi, bacteria, and bacterial spores while preserving the biologic and mechanical properties of the tissue. BioCleanse (Regeneration Technologies, Alachua, FL) is a fully automated, low-temperature, chemical sterilization process. Alternating cycles of vacuum and pressure remove endogenous donor material such as blood and lipids and completely perfuse the tissue with chemical sterilants validated to consistently eliminate bacteria, bacterial spores, fungi, and viruses.[24]

The purpose of our study was to evaluate the effect of this sterilization technique on the mechanical properties of soft-tissue allografts. Submaximal cyclical and load-to-failure tests were used to test the forces on BPTB allografts. The hypothesis was that there would be no significant effect of BioCleanse treatment on the mechanical properties evaluated by these tests.

METHODS

The BPTB specimens were harvested from 17 human cadaveric donors, machined, and prepared by Regeneration Technologies, according to the standard procedures of the company for donor consent and tissue procurement and treatment. The donor ages ranged from 19 to 88 years (mean, 52.5 years), and donors included 6 women and 11 men. Soft-tissue specimens were obtained as approximately 10-mm central third or hemi-BPTB units.

After tissue harvest, donor-matched specimens were randomly assigned to either the BioCleanse-treated group or untreated control group (20 specimens per group). Specimens in the untreated control group were aseptically processed tissue that had not been sterilized. The material properties of specimens in this group were unaltered, making the specimens ideal control samples. The specimens were stored at −80°C until they were shipped to our institution. Specimens arrived frozen and were stored at −20°C until being tested.

Specimens were thawed to room temperature on the day of mechanical testing and protected from desiccation by saline irrigation and moist gauze. Specimens were moistened only, not saturated. A 0.05-mm precision caliper was used to measure tendon length and width and thickness at 3 longitudinal locations. The 3 measurements of width and thickness were averaged to calculate the cross-sectional area.

Specimens were embedded in cylindrical fixtures using Hygenic Orthodontic Resin (Coltene/Whaledent, Cuyahoga Falls, OH), with stainless steel wires inserted in the middle of the tibial and patellar bone blocks to anchor them in the embedding material. The specimen and embedding fixtures were loaded into an environmental chamber on the servohydraulic testing machine (MTS Model 858; MTS Systems, Eden Prairie, MN). The specimen was protected from desiccation during mechanical testing by saline irrigation at 37°C in the environmental chamber.

Specimens were pretensioned at 89 N (20 pound force) and held in displacement control for 25 minutes. Tension was adjusted back to 89 N (20 pound force) at 5 minutes and 15 minutes. After 25 minutes, the specimen was unloaded to 30 N and held for 1 minute before commencing cyclic loading. Specimens were tested in force control under cyclic loading for 1,000 cycles (1 Hz) between 50 N and 250 N, followed immediately by ramping to failure at a rate of 50 mm/min. Cyclic creep was measured between cycles 3 and 1,000, stiffness was measured during the tenth cycle, and maximal load to failure was measured for each specimen. The mode of failure was also recorded for each specimen as midsubstance, insertion site, or at the bone plug.

The statistical analysis focused primarily on evaluating the effect of the treatment on the biomechanical properties of the tissue samples. Separate analyses of the BPTB specimens were performed for the outcomes of maximum force, ultimate stress, stiffness, and elongation. For each end-point, the effect of treatment on the outcome was analyzed with generalized linear models.[25] Because some cadavers contributed multiple specimens and these specimens were allocated within and between treatment groups, generalized estimating equations[26] were used in the models to properly account for the within-cadaver correlation and to correctly estimate the variance. If the effect on the outcome was significant when the age group classifications were analyzed, contrast statements were generated to produce pair-wise comparisons among the 3 age groups. To guard against an increased type I error rate, the P values were reported with a Bonferroni adjustment. All statistical tests were 2 sided, and adjusted P values $<.05$ were considered statistically significant.

RESULTS

Analysis of the donor-matched pairs showed no statistically significant difference between untreated and BioCleanse-treated specimens in stiffness, cyclic creep, maximum force, or ultimate stress (Table 1, online only; available at www.arthroscopyjournal.org). The cross-sectional area of the BioCleanse-treated specimens was significantly greater than that of the control specimens (67.9 ± 18.6 mm^2 v 60.8 ± 15.0 mm^2, respectively) ($P = .03$). Thus, although the average maximum force for the BioCleanse specimens was higher than that for the control specimens (but nonsignificant), the mean values were identical between the groups when normalized to the cross-sectional area (ultimate stress).

When the mechanical testing results were analyzed by sex, the specimens from women had a significantly higher cyclic creep (0.55 ± 0.46 mm v 0.28 ± 0.19 mm, $P = .03$) and ultimate stress (33.3 ± 12.5 mm v 26.1 ± 9.5 mm, $P = .05$) than specimens from men (Table 2, online only; available at www.arthroscopyjournal.org). However, this study was not powered to evaluate the effect of sex, and the sample sizes for women (n = 6) and men (n = 11) specimens were disproportionate.

Although the effect of age was not a primary focus of this study, specimens in the 15- to 40-year-old group (n = 6) and the 41- to 65-year-old group (n = 9) had significantly greater ultimate stress than those in the 66- to 90-year-old group (n = 5) (33.8 ± 10.0 MPa, 30.4 ± 11.7 MPa, and 20.7 ± 7.6 MPa, respectively, $P < .001$) (Table 3, online only; available at www.arthroscopyjournal.org).

There was no significant difference in location of failure between the 2 groups. In the untreated group, 8 specimens failed within the midsubstance, and 12 failed at the bone or bone-tendon insertion. In the BioCleanse-treated group, 11 specimens failed within the midsubstance, and 9 failed at the bone or bone-tendon insertion.

DISCUSSION

Several clinical studies that compared the long-term outcomes of allografts versus autografts for ACL reconstruction have suggested a higher rerupture rate in patients receiving an allograft.[4,27,28] However, other studies have shown a comparable rate of rerupture for allografts and autografts.[3,5] Forces sustained by the reconstructed ACL during most activities have been reported to be approximately 450 N.[29] Our study showed that the initial maximum force and ultimate stress of BioCleanse-treated specimens are not only comparable to those of untreated specimens but are sufficient to sustain the forces encountered by the knee during the currently recommended early intensive rehabilitation.

In addition to the risk of rerupture, potential problems after ACL reconstruction include increased knee laxity. Some clinical studies have shown that allografts and autografts have comparable laxity,[1,30] but other studies have shown slightly increased knee laxity with allografts.[2,5,30] Although the increased laxity reported for allografts may be caused by the delayed ligamentization in allografts compared with autografts,[31] laxity may also be affected by the effects of irradiation on collagen. Laboratory studies have shown a dose-related increase in cyclic creep in gamma-irradiated allografts.[20,23]

In the present study, static pretensioning at 89 N was chosen to simulate forces applied by the surgeon before and during implantation. Subsequent submaximal cyclical linear loading between 50 N and 250 N simulates forces encountered during implantation and early in rehabilitation. A previous study evaluating cyclic creep did not subject grafts to static pretensioning and showed greater elongation of grafts subjected to 1,000 cycles between 50 N and 250 N (4.4 ± 1.5 mm for grafts treated with 2 Mrad of irradiation and 3.4 ± 1.0 mm for untreated grafts) than our study did.[23] Our results are comparable to those of a previ-

ous study in which cyclic loading was evaluated after static pretensioning.[19] In that study, specimens treated with 4 Mrad of irradiation were compared with untreated specimens. Specimens underwent a static load of 90 N for 10 minutes, followed by 3,600 cycles between 50 N and 200 N. Cyclic creep averaged 0.5 ± 0.3 mm for the irradiated grafts and 0.4 ± 0.2 mm for the untreated grafts ($P > .5$). In our study, cyclic creep averaged 0.40 ± 0.26 mm for BioCleanse-treated specimens and 0.38 ± 0.42 mm for untreated specimens.

The effect of donor age on mechanical properties was not a primary aim of our study. However, our results correlate with and contribute to the findings of a previous study that evaluated the effect of age on the mechanical properties of BPTB specimens in which specimens from donors 29 to 50 years old had significantly greater maximum strength than those from donors 64 to 93 years old.[32] However, grafts from donors between the ages of 41 and 65 years in the current study do not appear to be significantly weaker than those from younger donors. This trend was also reported in a study involving allograft specimens from donors 18 to 55 years old in which no significant correlation was found between age and mechanical properties.[33] Therefore, although it is accepted that donor age will affect tissue mechanical properties, the apparent age threshold is unknown. In a pool already restricted by the number of donors and screening for infectious risk factors, the supply of donor tissue is limited. The ability to use tissue from older donors would increase the availability of tissue for use in clinical applications.

The present study has several limitations. The results represent the time zero, preimplantation, in vitro mechanical properties of the grafts. Further study is needed on the process and rate of biologic incorporation and ligamentization of BioCleanse-treated allograft specimens compared with autografts as well as untreated and irradiated allografts. Further study also is needed on the long-term clinical outcomes of patients receiving these grafts. In addition, because the cross-sectional area of the allografts could not be controlled in this study, there was a difference in area between the 2 groups. By normalizing the maximum force to the measured cross-sectional area, we attempted to minimize the mechanical strength bias that would be caused by graft size. However, it is possible that increased size may also influence the stiffness and decrease the relative creep because of an increased number of collagen cross-links.

Despite these limitations, our study has shown that soft-tissue grafts sterilized with the BioCleanse treatment process have mechanical properties comparable to those of untreated, fresh-frozen grafts. These results suggest that these chemically treated allografts initially possess mechanical properties sufficient for use in ACL reconstruction.

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TABLE 1. *Comparison of Mechanical Properties of Control and BioCleanse Groups**

	Group		
Property	Control	BioCleanse	*P* Value
Stiffness, N/mm	235.3 ± 37.6	222.3 ± 53.4	.37
Cyclic creep, mm	0.38 ± 0.42	0.40 ± 0.26	.81
Maximum force, N	1,685.7 ± 471.6	1,807.0 ± 657.8	.47
Ultimate stress, MPa	29.0 ± 9.8	29.0 ± 12.8	.98

*Values are mean ± standard deviation.

TABLE 2. *Comparison of Properties of Specimens by Sex**

Property	Men	Women	*P* Value
Stiffness, N/mm	235.5 ± 41.2	218.8 ± 52.4	.26
Cyclic creep, mm	0.28 ± 0.19	0.55 ± 0.46	.03 (F > M)
Maximum force, N	1,799.2 ± 586.1	1,667.1 ± 549.2	.43
Ultimate stress, MPa	26.1 ± 9.5	33.3 ± 12.5	.05 (F > M)

*Values are mean ± standard deviation.

TABLE 3. *Comparison of Properties of Specimens by Age Group**

	Age, yr			
Property	15-40	41-65	66-90	*P* Value
Stiffness, N/mm	215.3 ± 52.7	232.1 ± 43.8	239.1 ± 42.5	.20
Cyclic creep, mm	0.47 ± 0.35	0.43 ± 0.41	0.23 ± 0.13	.08
Maximum force, N	1,919.3 ± 667.6	1,752.6 ± 554.3	1,527.4 ± 425.6	.11
Ultimate stress, MPa	33.8 ± 10.0	30.4 ± 11.7	20.7 ± 7.6	<.001 (15-40 > 66-90) (41-65 > 66-90)

*Values are mean ± standard deviation.