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SYMPOSIUM: IMPROVING CARE FOR PATIENTS WITH ACL INJURIES: A TEAM APPROACH

What Factors Influence the Biomechanical Properties of Allograft Tissue for ACL Reconstruction? A Systematic Review

Drew A. Lansdown MD, Andrew J. Riff MD, Molly Meadows MD, Adam B. Yanke MD, Bernard R. Bach Jr MD

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Abstract

Background Allograft tissue is used in 22% to 42% of anterior cruciate ligament (ACL) reconstructions. Clinical outcomes have been inconsistent with allograft tissue, with some series reporting no differences in outcomes and others reporting increased risk of failure. There are numerous variations in processing and preparation that may influence the eventual performance of allograft tissue in ACL reconstruction. We sought to perform a systematic review to summarize the factors that affect the biomechanical properties of allograft tissue for use in ACL reconstruction. Many factors might impact the biomechanical properties of allograft tissue, and these should be understood when considering using allograft tissue or when reporting outcomes from allograft reconstruction.

Questions/purposes What factors affect the biomechanical properties of allograft tissue used for ACL reconstruction?

Methods We performed a systematic review to identify studies on factors that influence the biomechanical properties of allograft tissue through PubMed and SCOPUS databases. We included cadaveric and animal studies that reported on results of biomechanical testing, whereas studies on fixation, histologic evaluation, and clinical outcomes were excluded. There were 319 unique publications identified through the search with 48 identified as relevant to answering the study question. For each study, we recorded the type of tissue tested, parameters investigated, and the effects on biomechanical behavior, including load to failure and stiffness. Primary factors identified to influence allograft tissue properties were graft tissue type, sterilization methods (irradiation and chemical processing), graft preparation, donor parameters, and biologic adjuncts.

Results Load to failure and graft stiffness varied across different tissue types, with nonlooped tibialis grafts exhibiting the lowest values. Studies on low-dose irradiation showed variable effects, whereas high-dose irradiation consistently produced decreased load to failure and stiffness values. Various chemical sterilization measures were also associated with negative effects on biomechanical properties. Prolonged freezing decreased load to failure, ultimate stress, and ultimate strain. Up to eight freeze-thaw cycles did not lead to differences in biomechanical properties of cadaveric grafts. Regional differences were noted in patellar tendon grafts, with the central third showing the highest load to failure and stiffness. Graft diameter strongly contributed to load-to-failure measurements. Age older than 40 years, and especially older than 65 years, negatively impacted biomechanical properties, whereas gender had minimal effect on the properties of allograft tissue. Biologic adjuncts show potential for improving in vivo properties of allograft tissue.

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Conclusions Future clinical studies on allograft ACL reconstruction should investigate in vivo graft performance with standardized allograft processing and preparation methods that limit the negative effects on the biomechanical properties of tissue. Additionally, biologic adjuncts may improve the biomechanical properties of allograft tissue, although future preclinical and clinical studies are necessary to clarify the role of these treatments.

Clinical Relevance Based on the findings of this systematic review that emphasize biomechanical properties of ACL allografts, surgeons should favor the use of central third patellar tendon or looped soft tissue grafts, maximize graft cross-sectional area, and favor grafts from donors younger than 40 years of age while avoiding grafts subjected to radiation doses > 20 kGy, chemical processing, or greater than eight freeze-thaw cycles.

Introduction

Recent estimates show 22% to 42% of anterior cruciate ligament (ACL) reconstructions are performed with allograft tissue [10, 69]. The rationale for the use of allograft tissue includes shorter operative time, improved cosmesis, predictable tissue size, and decreased donor site morbidity and postoperative pain [30]. Systematic reviews on clinical outcomes aimed at comparing allograft and autograft techniques demonstrate no differences in outcomes across all age groups [11, 26, 44].

However, large cohort studies have identified the use of allograft in younger patients as a risk factor for graft failure, leading to recommendations for autograft use in younger patients in both primary and revision ACL reconstruction [39, 41, 43, 50]. The graft selected for ACL reconstruction must be able to withstand the biomechanical forces encountered by the native ACL. Previous studies have defined the properties of the normal ligament in patients aged 16 to 35 years as ultimate strength from 1730 to 2160 N and stiffness of 182 to 242 N/mm [47, 72]. A linear age-related decline in these properties has been observed with values decreasing to 734 to 1503 N and 182 to 220 N/mm, respectively, in older individuals [47, 72]. Differences in native ACL properties provide a rationale for stratifying graft choice based on patient age. Many factors may impact the integrity of allografts, including irradiation dose, graft type, and donor characteristics, and grafts are provided by multiple different tissue banks that utilize various, often proprietary, preparation techniques [2]. The ideal graft type and preparation strategy remain controversial.

To make appropriate recommendations to patients before ACL reconstruction, surgeons must understand the variables that influence the biomechanical properties of allograft tissue. We therefore sought to answer the following question by performing a systematic review: What factors affect the biomechanical properties of allograft tissue used for ACL reconstruction?

Materials and Methods

We utilized PubMed and SCOPUS databases to perform searches for relevant published studies on parameters that affect the biomechanical properties of allograft tissue for ACL reconstruction. A search was performed in September 2016 with the following search terms: (allograft OR allografts) AND ("anterior cruciate ligament" OR "ACL") AND (biomechanics OR biomechanical). There were 204 results from the PubMed search and 287 from the SCOPUS search with 319 representing unique publications. Before evaluating the results, this review was registered with PROSPERO in accordance with recommendations from PRISMA. Only English-language studies were included, and abstracts and conference proceedings were excluded. Inclusion criteria consisted of articles containing an investigation of potential variables that impact allograft biomechanical properties and reporting of biomechanical testing data. Articles on fixation properties and devices, histologic or biochemical evaluations, or those that lacked investigation of properties of allograft tissue were excluded as well as review articles.

Article titles and abstracts were reviewed independently by two authors (DAL, AJR) for appropriateness to include in the review with 94 studies selected for evaluation based on their titles. Data extraction was performed by one author (DAL) and reviewed by a second (AJR) to maintain consistency with differences adjudicated based on consensus. A standardized form was used for data collection. There were 48 remaining studies that were included in this systematic review (Fig. 1).

Studies were grouped based on various factors investigated in each study. There were six primary categories identified: graft type, sterilization methods (including irradiation and chemical processing), preservation methods, graft preparation, donor factors, and biologic adjuncts. The study type was classified as human cadaveric, in vivo animal study, or in vitro animal study. Finally, the graft type (anatomic site) investigated in each study was recorded.

The included studies consisted of 32 human cadaveric studies, 10 in vivo animal studies, five in vitro animal studies, and one combined in vivo/in vitro animal study. Different graft types were compared in seven human

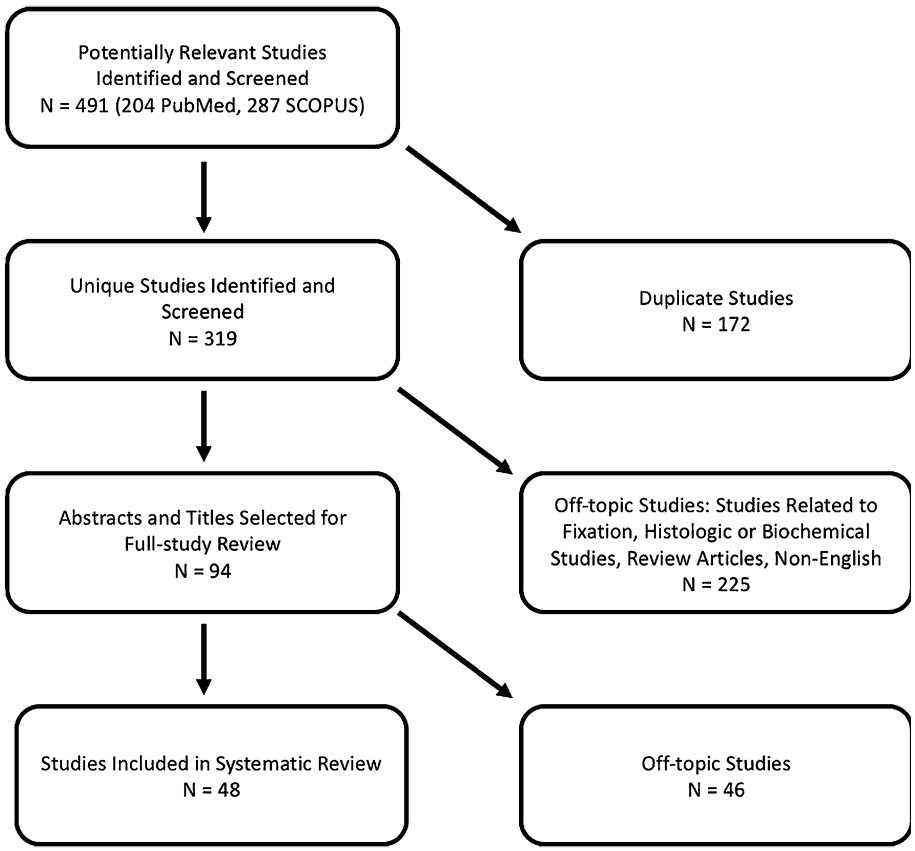

Fig. 1 A flowchart of the systematic review process shows the number of articles reviewed at each time point and those included in the final study group.

cadaveric studies and included evaluation of bone patellar-tendon bone (BPTB) allograft (N = 4), tibialis anterior (N = 5), tibialis posterior (N = 4), peroneal tendons (N = 2), quadriceps tendon (N = 2), hamstring tendons (N = 1), and iliotibial band/fascia lata (N = 2) [1, 15, 21, 33, 42, 51, 60]. Radiation was evaluated in 18 studies [5–7, 19, 25, 29, 31, 32, 34–36, 45, 56–59, 70, 74], including four studies on low-dose gamma irradiation (up to 20 kGy) [7, 19, 31, 74], three studies on high-dose gamma irradiation (20–40 kGy) [5, 25, 29], and four studies on the dose-dependent effects of gamma irradiation [6, 25, 29, 45]. Electron beam (E-beam) irradiation was evaluated in six studies [32, 34–36, 56, 57]. Two studies evaluated the use of a radioprotectant to limit damage during treatment with irradiation [59, 70]. Chemical processing was investigated in seven studies [5, 23, 24, 37, 53–55], including peracetic acid in three studies [23, 53, 54], BioCleanse® (RTI Surgical, Inc, Alachua, FL, USA) treatment, an automated low-temperature chemical sterilization process, in two studies [37, 55], ethylene oxide sterilization in one study [24], and supercritical carbon dioxide treatment in one study [5]. Preservation methods were evaluated in eight studies [17, 28, 32, 38, 48, 64, 66, 77]. The effect of freezing was evaluated in two studies [28, 64]. The impact of freeze-thaw cycles was investigated in three studies [17, 38, 66]. Three studies evaluated glycerol preservation of allografts [32, 64, 77] and two on cryoprotectants [48, 64]. Graft preparation was evaluated in seven studies [1, 9, 18, 46, 61, 75, 76], including three studies on preparation factors for BPTB grafts [46, 75, 76] and two on preparation of tibialis tendons [1, 18]. Two studies investigated the effects of graft diameter on mechanical properties [9, 61]. Donor age was evaluated in four cadaveric studies [8, 31, 37, 67], and sex differences were investigated in one study [37]. The impact of biologic adjuncts on eventual biomechanical studies was reported in two in vitro animal studies [16, 71].

Results

Graft Type

There was large variation in load to failure (LTF) and stiffness for different graft types (Fig. 2). LTF was lowest in nonlooped tibialis anterior/tibialis posterior (TA/TP) tendons (777–789 N) [1], whereas the highest LTF was observed in looped TA/TP tendons (3012–4112 N) [15, 33, 51]. The lowest stiffness was observed in non-looped TA/TP (61–73 N/mm) [1], and the highest stiffness

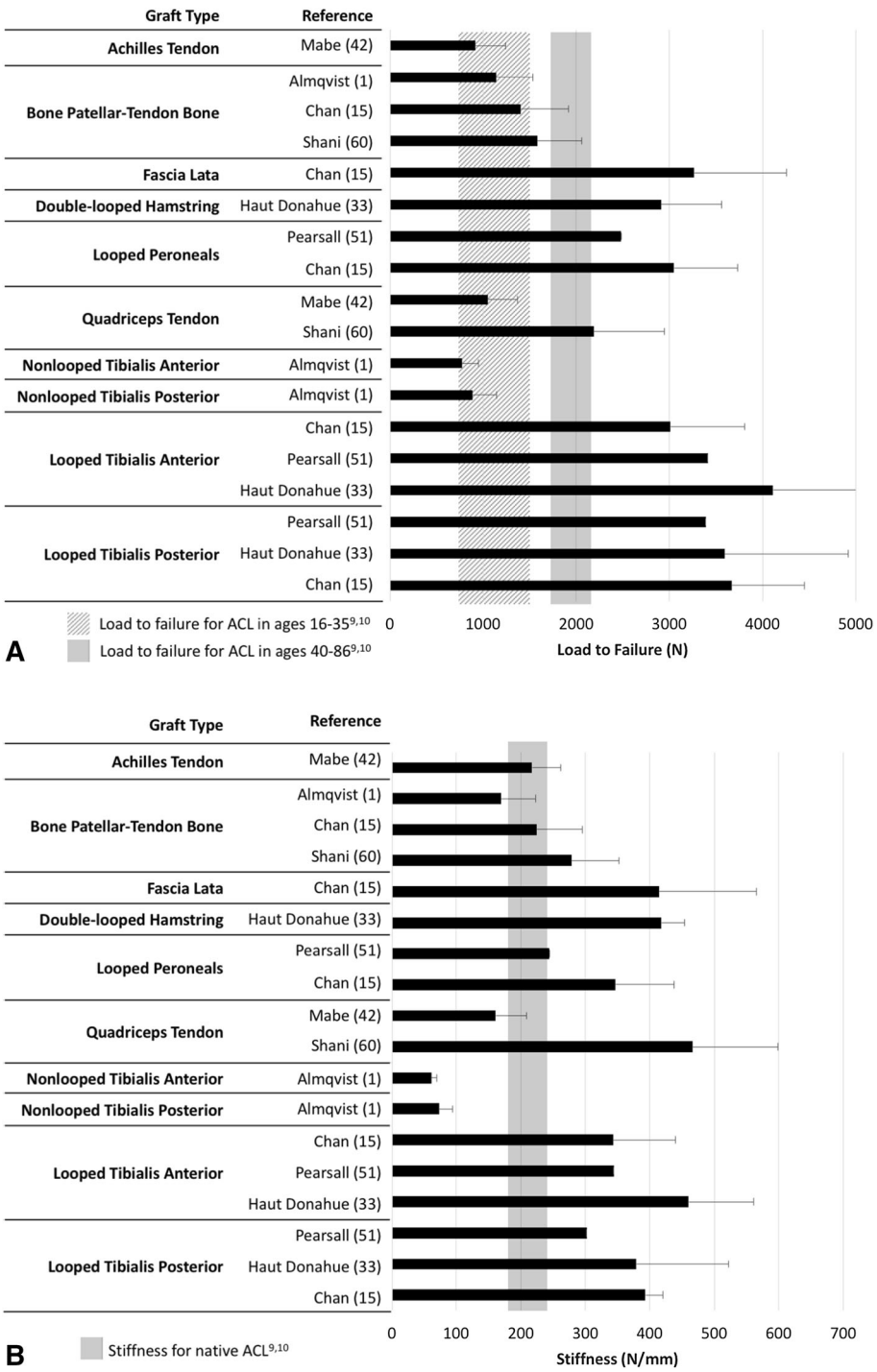

Fig. 2A–B There is great variability in the reported biomechanical properties of various graft types used in ACL reconstruction, including (**A**) LTF and (**B**) stiffness. The mean LTF (with error bars showing SD) is shown relative to previously reported normal values of 1730 to 2160 N for ages 16 to 35 years (striped box) and 734 to 1503 N for ages 40 to 86 years (solid gray) [48, 73]. The mean stiffness values (with error bars showing SD) are shown relative to reported values of 182 to 242 N/mm for ages 16 to 35 years (solid gray), which encompass the reported values of 182 to 220 N/mm in ages 40 to 86 years [48, 73].

values were noted in quadriceps tendon grafts (161–466.2 N/mm) [42, 60].

Irradiation

Low-dose gamma irradiation ($\leq$ 20 kGy) had mixed effects on biomechanical properties, ranging from a 20% reduction in stiffness with 10 to 12 kGy [74] and 20% reduction in LTF with 20 kGy [19] to no difference in biomechanical properties after treatment with 12 to 18 kGy (Table 1) [7, 31]. A dose-dependent relationship was observed with higher levels of gamma irradiation (20–40 kGy) consistently linked to decreased LTF (54%–74% of nonirradiated tissue) [5, 6, 25, 29, 35, 36, 45, 58]. Stiffness was decreased in five of six studies testing these levels of irradiation,

Table 1. Studies on the effects of irradiation on allograft tissue properties

Study	Study type	Time of testing	Graft types	Number	Parameters tested	Key findings
Yanke et al. [74]	Cadaveric	T0	BPTB	14	GI (10–12 kGy) versus NIT	GI decreased stiffness by 20% ($p = 0.035$); no difference for maximum load, maximum stress, and strain
Curran et al. [19]	Cadaveric	T0	BPTB	26	GI (20 kGy) and Allowash (LifeNet Health, Tampa, FL, USA) versus Allowash alone	LTF (80% of NIT; $p = 0.007$) and graft elongation (130%; $p = 0.03$) were negatively affected by irradiation
Bhatia et al. [7]	In vivo, rabbits	2 weeks, 8 weeks	HS	58	GI (12 kGy), NIT, and autograft	No differences observed at 8 weeks among autograft, NIT, and 12 kGy for maximum load or stiffness
Greaves et al. [31]	Cadaveric	T0	TA/TP	126	GI (14.6–18.0 kGy) versus NIT	No difference for LTF, stiffness, displacement at failure, and failure stress
Baldini et al. [5]	Cadaveric	T0	TA/TP	38	GI (20–28 kGy) versus NIT	No differences for failure stress or LTF
Balsly et al. [6]	Cadaveric	T0	BPTB, TA, FL, semi-T	76	GI (18.3–21.8 kGy and 24.0–28.5 kGy) versus NIT	24.0–28.5 kGy decreased tensile strength for BPTB (72% of NIT; $p = 0.016$); 18.3–21.8 kGy did not impact strength or modulus
Fideler et al. [25]	Cadaveric	T0	Hemi-BPTB	60	GI (20 kGy, 30 kGy, 40 kGy) versus NIT	Dose-dependent reduction in stiffness (20 kGy: 91% of NIT, $p = 0.11$; 30 kGy: 84% of NIT, $p = 0.002$; 40 kGy: 54% of NIT, $p < 0.0001$) and LTF (20 kGy: 85% of NIT, $p = 0.002$; 30 kGy: 78% of NIT, $p < 0.0001$; 40 kGy: 54% of NIT, $p < 0.0001$)
Gibbons et al. [29]	In vitro, goat	T0	BPTB	48	GI (20 kGy and 30 kGy) versus NIT	30 kGy reduced stiffness (83% of NIT, $p < 0.005$), maximum force (73%, $p < 0.05$), maximum stress (85%, $p < 0.01$); no differences for 20 kGy versus NIT
Hoburg et al. [35]	Cadaveric	T0	BPTB	44	GI (34 kGy), single E-beam (34 kGy), fractionated E-beam (34 kGy), NIT	LTF decreased for GI (56% of NIT, $p < 0.001$) and single-dose E-beam (68%, $p = 0.002$); no difference between NIT and fractionated E-beam (89%; $p = 0.27$)
Hoburg et al. [36]	Cadaveric	T0	BPTB	50	GI (25 and 34 kGy) versus E-beam irradiation (25 and 34 kGy)	LTF lower in GI-25 kGy (58% of NIT) and GI-34 kGy (62%) and E-beam-34 kGy (65%; $p < 0.005$); stiffness was lower in GI-25 kGy (63% of NIT) and GI-34 kGy (85%); no difference between E-beam 25 kGy and E-beam 34 kGy
McGilvray et al. [45]	In vitro, sheep	T0	BPTB	64	GI (15 kGy and 25 kGy) versus NIT	LTF decreased in 25 kGy (69% versus NIT; $p = 0.027$) and ultimate strength (71%; $p = 0.035$); no differences for 15 kGy versus NIT
Schwartz et al. [58]	In vivo, goat	6 mo	BPTB	31	GI (40 kGy) versus NIT	Decreased linear stiffness (70% of nonirradiated value; $p < 0.05$) and LTF (79%; $p < 0.05$) with GI
Gut et al. [32]	Cadaveric	T0	BPTB	50	FF and GI (25 kGy, 35 kGy, 50 kGy, 100 kGy) versus glycerolization + GI (35 kGy) versus lyophilization + GI (35 kGy)	Dose-dependent decrease in LTF with increasing GI (25 kGy: 85% of NIT; 35 kGy: 79%; 100 kGy: 68%); glycerolization and lyophilization + GI resulted in decrease of 40%-50% of LTF relative to NIT
Hoburg et al. [34]	Cadaveric	T0	BPTB	32	E-beam irradiation (15, 25, and 34 kGy) versus NIT	LTF decreased in 34 kGy (80% of NIT; $p = 0.036$); no differences strain, cyclic elongation, or stiffness

Table 1. continued

Study	Study type	Time of testing	Graft types	Number	Parameters tested	Key findings
Schmidt et al. [56]	In vivo, sheep	6 weeks, 12 weeks	FDS	36	Fractionated E-beam (8 × 3.4 kGy) versus NIT	LTF was lower at 6 weeks (13% of NIT) and 12 weeks (43%)
Wei et al. [70]	Cadaveric	T0	FDS	40	Single E-beam (50 kGy), fractionated E-beam (50 kGy), radioprotectant + fractionated E-beam (50 kGy), and NIT	50 kGy E-beam had detrimental effects on LTF (70% of NIT; $p = 0.012$); no difference in LTF between fractionated E-beam and radioprotectant + fractionated E-beam versus NIT
Schmidt et al. [57]	In vivo, sheep	6 weeks, 12 weeks	FDS	24	E-beam irradiation (34 kGy) versus nonirradiated allograft	E-beam irradiated grafts showed decreased LTF (21% of NIT) and stiffness (18%) at 12 weeks after ACL reconstruction
Seto et al. [59]	In vivo, sheep	12 weeks, 24 weeks	Achilles	24	GI (50 kGy), radioprotectant + GI (50 kGy), and NIT	No differences observed between tissue treated with radioprotectant and NIT

T0 = time zero; BPTB = bone patellar-tendon bone; HS = hamstring; TA = tibialis anterior; TP = tibialis posterior; FL = fascia lata; semi-T = semitendinosus; FDS = flexor digitorum superficialis; GI = gamma irradiation; NIT = nonirradiated tissue; FF = fresh-frozen; LTF = load to failure.

ranging from 54% to 85% of values for nonirradiated tissue [25, 29, 35, 36, 58], although one study showed no difference in stiffness in sheep BPTB at time zero [45].

E-beam irradiation > 25 kGy led to detrimental effects on structural properties, including 79% LTF for 35 kGy compared with nonirradiated tissue and 68% LTF with 100 kGy [32, 34]. Studies on fractionation of E-beam irradiation reported a negative impact on LTF (21%–89% of nonirradiated allografts) [56, 70]. Stiffness was no different after treatment with fractionated E-beam irradiation (multiple smaller doses of irradiation rather than one single, higher dose) compared with nonirradiated cadaveric BPTB tendons [36], although stiffness was 18% of fresh-frozen allografts in an in vivo sheep model at 12 weeks after reconstruction [57]. In comparing E-beam with gamma irradiation, gamma irradiation produced decreased values for LTF (81%–94% of E-beam values) and stiffness (82%–88%) [36]. Radioprotectants (either 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide) and N-hydroxyl succinimide and a free-radical scavenger or ascorbate had a protective effect when treating tissue with high-dose irradiation with no differences observed for stiffness and LTF for both sheep Achilles tendon grafts and cadaveric flexor digitorum superficialis tendons [59, 70].

Chemical Sterilization

Peracetic acid showed mixed effects across three studies, ranging from a 39% decrease in LTF in sheep at 12 weeks after ACL reconstruction [53] to no difference in stiffness or LTF in cadaveric BPTB grafts [54] to a 48% increase in LTF in rabbits at 12 weeks after ACL reconstruction (Table 2) [23]. BioCleanse® (RTI Surgical, Inc) had no effect on LTF, ultimate stress, or cyclic loading relative to untreated specimens [37, 55]. Ethylene oxide sterilization was associated with decreased maximum force (29% of untreated, $p < 0.001$) and decreased graft stiffness (43%, $p < 0.001$) in goats at 6 and 12 months after BPTB allograft reconstruction [24]. Supercritical CO_2 treatment also led to lower stiffness than unprocessed (27% of untreated) and irradiated grafts (36%) in cyclic testing [5].

Preservation Methods

Freezing at −80° C for 30 days to 9 months led to decreased ultimate load (82% of fresh tendon value; $p < 0.05$) [28], decreased ultimate stress (70%, $p < 0.05$) [28], and variable effects on stiffness (71%–115%) [28, 64] (Table 3). There were mixed effects of multiple freeze-thaw cycles [17, 38, 66]. Two studies that evaluated BPTB allografts showed no difference in any measured property,

Table 2. Studies on the effects of chemical sterilization on allograft tissue properties

Study	Study type	Time of testing	Graft types	Number	Parameters tested	Key findings
Scheffler et al. [53]	In vivo, sheep	6 weeks, 12 weeks	FDS	16	Peracetic acid-sterilization versus FFA versus autograft versus native ACL	LTF (38% of FFA; $p < 0.05$) and stiffness (64%; $p < 0.05$) decreased versus FFA at 12 weeks
Scheffler et al. [54]	Cadaveric	T0	BPTB	16	Peracetic acid ethanol sterilization versus FFA	No differences in strain, creep, stiffness, LTF, or maximum elongation
Dong et al. [23]	In vitro/in vivo, rabbit	T0, 12 weeks	Semi-T	130	Chemical decellularization with peracetic acid versus FFA	No differences for LTF or stiffness at T0; increased LTF for decellularized group at 12 weeks (148% of FFA, $p = 0.02$)
Jones et al. [37]	Cadaveric	T0	BPTB	40	BioCleanse® (RTI Surgical, Inc, Alachua, FL, USA) versus untreated tissue	No differences in stiffness, maximum force, creep, or ultimate stress between BioCleanse-treated and untreated tissue
Schimizzi et al. [55]	Cadaveric	T0	TA	36	BioCleanse® sterilization versus FF versus GI (20–26 kGy)	No differences for creep, stiffness in cyclic loading, or LTF; stiffness in first cycle higher for BioCleanse (125% versus FFA) and GI (123%, $p < 0.005$)
Drez et al. [24]	In vivo, goat	26 weeks, 52 weeks	BPTB	24	Freeze-dried, ethylene oxide-sterilization versus native ACL	LTF (43% of native ACL, $p < 0.001$) and stiffness (29% of native ACL, $p < 0.001$) negatively impacted by sterilization
Baldini et al. [5]	Cadaveric	T0	TA/TP	38	$SCCO_2$ versus gamma irradiation (20–28 kGy) versus NIT	$SCCO_2$ treatment had 27%-36% lower stiffness versus NIT and irradiated tendons

T0 = time zero; FDA = flexor digitorum superficialis; BPTB = bone patellar-tendon bone; semi-T = semitendinosus; TA = tibialis anterior; TP = tibialis posterior; FFA = fresh-frozen allograft; ACL = anterior cruciate ligament; GI = gamma irradiation; $SCCO_2$ = supercritical carbon dioxide; NIT = nonirradiated tissue; LTF = load to failure.

Table 3. Studies on the effects of preservation methods on allograft tissue properties

Study	Study type	Time of testing	Graft types	Number	Parameters tested	Key findings
Giannini et al. [28]	Cadaveric	T0	TP	22	Fresh-frozen at −80° C for 30 days versus fresh allograft	LTF (82% relative to fresh allograft; $p < 0.05$), ultimate stress (70%; $p < 0.05$), and ultimate strain (66%; $p < 0.05$) all decreased in fresh-frozen versus fresh allograft
Suhodolčan et al. [64]	Cadaveric	T0	BPTB	70	Frozen (3, 6, and 9 months), cryopreserved in 10% glycerol (3, 6, and 9 months), and fresh	Higher elongation rates observed in allografts frozen for 3 months versus fresh specimens (128% of fresh value; $p = 0.03$); no differences observed in elongation rates for cryopreserved groups
Chen et al. [17]	In vitro, rabbit	T0	Achilles	21	One versus 3 versus 10 freeze-thaw cycles	3 and 10 cycles decreased maximum load (76% for both 3 and 10 cycles versus 1; $p < 0.05$), energy of maximum load (67% for both versus 1; $p < 0.05$), and maximum stress (74% for 3, 80% for 10; $p < 0.05$)
Jung et al. [38]	Cadaveric	T0	BPTB	63	One versus 4 versus 8 freeze-thaw cycles	No differences observed for LTF, stiffness, creep, or mode of failure
Suto et al. [66]	In vitro, rats	T0	BPTB	24	Frozen at −80° C for 3 weeks versus 5 freeze-thaw cycles versus fresh	No differences observed for ultimate stress, Young's modulus, or strain at failure
Gut et al. [32]	Cadaveric	T0	BPTB	50	FF and GI (25 kGy, 35 kGy, 50 kGy, 100 kGy) versus glycerolization + GI (35 kGy) versus lyophilization + GI (35 kGy)	Dose-dependent decrease in LTF with increasing GI (25 kGy: 85% of NIT; 35 kGy: 79%; 100 kGy: 68%); glycerolization and lyophilization + GI resulted in decrease of 40%-50% of LTF relative to NIT
Zimmerman et al. [77]	In vivo, sheep	6 months	BPTB	21	Chloroform-methanol (CM) treatment, propylene glycol/glycerol monolaurate treatment (PG), FFA and native ACL	LTF and stiffness decreased for fresh-frozen (LTF: 56%, $p < 0.10$; stiffness 70%, $p < 0.10$), CM treatment (LTF: 45%, $p < 0.05$; stiffness 45%, $p < 0.05$), and PG treatment (LTF: 30%, $p < 0.05$; stiffness 43%, $p < 0.05$) versus normal ACL
Nyland et al. [48]	Cadaveric	T0	TA	30	Incubation with cryoprotectant (8 hours versus 2 hours) versus fresh allograft	Stiffness at LTF decreased in both 8-hour (83% versus fresh allograft) and 2-hour (81%) groups ($p = 0.003$); no differences in LTF, yield load, or displacement during LTF

T0 = time zero; TP = tibialis posterior; BPTB = bone patellar-tendon bone; TA = tibialis anterior; FF = fresh-frozen; Gl = gamma irradiation; FFA = fresh-frozen allograft; ACL = anterior cruciate ligament; LTF = load to failure; NIT = nonirradiated tissue.

including LTF, stress, and stiffness, for up to eight freeze-thaw cycles [38, 66]. Chen et al. [17], however, reported a decrease in maximum load after three and 10 cycles (both 76% of one-cycle value, $p < 0.05$) for Achilles allografts.

Glycerolization and lyophilization before irradiation produced a 40% to 50% decrease in LTF [32], whereas peak load and stiffness were decreased after treatment of allografts with either propylene glycol and glycerol monolaurate (peak load 30%, stiffness 43% of normal ACL; $p < 0.05$) or chloroform-methanol extraction (peak load and stiffness 45% of normal ACL; $p < 0.05$) [77]. Incubation with a cryoprotectant for 2 to 8 hours resulted in a 17% to 19% decrease in stiffness but did not impact LTF [48]. Glycerol as a cryoprotectant to preserve cadaveric BPTB grafts for 3 to 9 months showed no difference in ultimate stress (112%–121% of fresh allograft) and ultimate stiffness (104%–115%) compared with fresh allograft [64].

Graft Preparation

The central third of the patellar tendon was biomechanically stronger than the medial third (LTF 61% [p = 0.002], stiffness 72% [p = 0.02] relative to the central third), lateral third (LTF 54% [p = 0.03], stiffness 62% [p = 0.001]), medial hemipatellar (LTF 69% [p = 0.006], stiffness 77% [p = 0.007]), or lateral hemipatellar (LTF 69% [p = 0.007], stiffness 78% [p = 0.008]) tendon grafts (Table 4) [75, 76]. A T-block modification, which may allow the use of patellar tendons longer than 50 mm, showed no difference for LTF with a 10-mm or 15-mm T-block, although stiffness was lower in the 15-mm T-block (79% of standard BPTB graft; p = 0.02) [46].

Looped tibialis grafts had a 75% to 100% increase in LTF ($p < 0.001$) and 220% to 287% increase in stiffness ($p < 0.001$) relative to nonlooped grafts [1]. Longitudinally splitting a TA allograft for double-bundle ACL reconstruction showed no difference in stiffness compared with an intact graft [18].

Graft diameter consistently affected mechanical properties for bone and soft tissue grafts. For hamstring grafts ranging from 6 to 9 mm in diameter, the LTF was increased for 7-mm (138% of 6-mm value; p = 0.01), 8-mm (166%; p = 0.01), and 9-mm (285%; p = 0.01) grafts [9]. For 4- to 4.5-mm and 8- to 9-mm BPTB grafts, the maximum load was 93% higher in the wider graft group [61].

Donor Parameters

Sex had minimal effect on graft properties, with no difference between male and female grafts for maximum force or stiffness, but male grafts showed decreased cyclic creep (51% of female value, p = 0.03) and ultimate stress (78%, p = 0.05) (Table 5) [37].

Increasing age had a negative correlation with mechanical properties [8, 31, 37, 67]. Weak correlations between age and ultimate tensile strength ($r^2 = 0.063$, $p < 0.001$) [67] and modulus of elasticity ($r^2 = 0.11$, $p < 0.05$) [8] were reported. Donors older than 65 years of age had ultimate stress values that were 61% of stress for ages 15 to 40 years ($p < 0.001$) and 68% of that for ages 41 to 65 years ($p < 0.001$) [37].

Biologic Adjuncts

Vascular endothelial growth factor (VEGF) and transforming growth factor β (TGFβ-1)-transduced bone mesenchymal stem cells produced the highest ultimate failure load and stiffness relative to VEGF or TGFβ-1 transduction alone or untreated grafts at 24 weeks after allograft ACL reconstruction in rabbits (Table 6) [16]. VEGF and sodium hyaluronate allografts had higher ultimate failure at 4 and 8 weeks after BPTB reconstruction in rabbits [71].

Discussion

The use of allograft for ACL reconstruction was initially reported in 1986 and has been adopted broadly as a result of diminished donor site morbidity, shortened operative time, graft availability in the revision setting, and reduced risk of arthrofibrosis [49]. A 2013 American Orthopaedic Society for Sports Medicine (AOSSM) survey revealed that allografts are used for 27% of ACL reconstructions and 62% of revision reconstructions [2]. Despite their abundant use, allografts have witnessed increased scrutiny in the last 5 years as a result of studies suggesting higher failure rates in young patients [39]. The rationale for this systematic review was to determine factors that optimize the biomechanical properties of allografts used for ACL reconstruction to help surgeons make informed choices about allograft selection and to further improve results of allograft reconstruction. Specifically, we identified and described the effects of graft type, sterilization (irradiation and chemical processing) and preservation, graft preparation, donor characteristics, and biologic adjuncts on graft strength and stiffness. There is notable variation in strength and stiffness among allograft tendons harvested from different sites, although most meet or exceed the ultimate tensile strength of the native ACL. Moderate-dose irradiation and chemical processing have detrimental effects on biomechanical properties of tissue and should be carefully scrutinized in clinical practice. Studies are mixed on low-

Table 4. Studies on the effects of graft preservation factors on allograft tissue properties

Study	Study type	Time of testing	Graft types	Number	Parameters tested	Key findings
Almqvist et al. [1]	Cadaveric	T0	BPTB, TA, TP	64	BPTB versus single-strand TA/TP versus looped TA/TP	Looped TA/TP had LTF (1553 N) and stiffness (236 N/mm) relative to BPTB (LTF: 1139 N; stiffness 169 N/mm; $p < 0.001$ for both versus looped) and single-strand TA/TP (LTF: 777–889 N; stiffness: 61–73 N/mm; $p < 0.001$ for both versus looped)
Boniello et al. [9]	Cadaveric	T0	Gracilis, semi-T	44	Graft diameters of 6, 7, 8, and 9 mm	LTF increased with larger diameter (6 mm–2358.8 N; 7 mm–3263.5 N; 8 mm–3907.8 N; 9 mm–4360.3 N; $p = 0.01$ for 6 versus 7, 8, and 9 mm)
Clark et al. [18]	Cadaveric	T0	TA	14	Whole TA versus split TA	LTF significantly lower in whole TA (74% relative to split TA; $p < 0.01$); no difference in absorbed energy
Nasert et al. [46]	Cadaveric	T0	BPTB	30	Standard bone plugs versus 10-mm T-block versus 15-mm T-block	Stiffness decreased in 15-mm T-block (79% versus standard; p = 002); no differences for 10-mm T-block versus standard plug
Shino et al. [61]	In vivo, dogs	30 weeks, 52 weeks	BPTB	32	4- to 4.5-mm allograft at 30 weeks versus 8- to 9-mm allograft at 30 weeks versus 8- to 9-mm allograft at 52 weeks versus 4- to 4.5-mm autograft at 30 weeks	Maximum load was lower in 4- to 4.5-mm allograft group at 30 weeks (125.1 N) relative to 8- to 9-mm allograft at 30 weeks (244.8 N) or 8- to 9 -m allograft at 52 weeks (227.1 N)
Yanke et al. [75]	Cadaveric	T0	BPTB	10	Central versus medial versus lateral third patellar tendon grafts	Central third had highest maximum load (central = 1680 N versus medial = 1033 N, $p = 0.002$; lateral = 908 N; $p = 0.027$)
Yanke et al. [76]	Cadaveric	T0	BPTB	9	Central third versus hemipatellar tendon grafts	Central third biomechanically superior to medial and lateral hemipatellar tendon grafts for maximum load (central: 2293 N; lateral: 1585 N; medial: 1575 N; $p < 0.01$ for central versus lateral and central versus medial) and linear stiffness (central: 356 N/mm; lateral: 277 N/mm; medial: 275 N/mm; $p < 0.01$ for central versus lateral and central versus medial)
Almqvist et al. [1]	Cadaveric	T0	BPTB, TA, TP	64	BPTB versus single-strand TA/TP versus looped TA/TP	Looped TA/TP had LTF (1553 N) and stiffness (236 N/mm) relative to BPTB (LTF: 1139 N; stiffness 169 N/mm; $p < 0.001$ for both versus looped) and single-strand TA/TP (LTF: 777–889 N; stiffness: 61–73 N/mm; $p < 0.001$ for both versus looped)

T0 = time zero; BPTB = bone patellar-tendon bone; TA = tibialis anterior; TP = tibialis posterior; semi-T = semitendinosus; LTF = load to failure; LTF = load to failure.

Table 5. Donor parameters

Study	Study type	Time of testing	Graft types	Number	Parameters tested	Key findings
Jones et al. [37]	Cadaveric	T0	BPTB	40	Young (15–40 years), middle age (41–65 years), old (66–90 years), and sex	Ultimate stress was decreased for old donors by 39% versus young donors and 32% versus middle-aged donors ($p < 0.001$)
Blevins et al. [8]	Cadaveric	T0	BPTB	82	Donor age from 17–54 years	No correlation between tensile strength and age; negative relationship between modulus elasticity and age ($r^2 = 0.11$, $p < 0.05$)
Greaves et al. [31]	Cadaveric	T0	TA/TP	126	Young (20–45 years), middle-aged (46–55 years), and old (56–65 years) donors	Age showed no significant effect on failure load, stiffness, failure stress, or displacement at failure
Swank et al. [67]	Cadaveric	T0	TP	550	6 age groups: 15–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, 70–79 years	Weak correlation of age and mechanical properties; best association was ultimate tensile strength ($r^2 = 0.063$; $p < 0.01$)

T0 = time zero; BPTB = bone patellar-tendon bone; TA = tibialis anterior; TP = tibialis posterior.

Table 6. Biologic adjuncts

Author	Study type	Time of testing	Graft types	Number	Parameters tested	Key findings
Chen et al. [16]	In vivo, rabbits	2, 4, 8 weeks	BPTB	90	Allograft treated with VEGF and SH versus VEGF alone versus SH alone versus buffer versus intact ACL	Ultimate failure at 2 weeks was decreased in VEGF/SH group relative to other groups; then was increased relative to others at 4 and 8 weeks
Wei et al. [71]	In vivo, rabbits	3, 6, 12, 24 weeks	Achilles	176	Graft treated with BMSCs transduced with VEGF, TGFβ-1, or TGFβ-1 + VEGF versus untreated	At 24 weeks, TGFβ-1/VEGF combined group had highest ultimate failure and stiffness; at 6, 12, and 24 weeks after surgery, LTF and stiffness were improved for the TGFβ-1 group alone relative to control ($p < 0.05$)

BPTB = bone patellar-tendon bone; VEGF = vascular endothelial growth factor; SH = sodium hyaluronate; ACL = anterior cruciate ligament; BMSCs = bone mesenchymal stem cells; TGFβ-1 = transforming growth factor β; LTF = load to failure.

dose irradiation (10–12 kGy), with some studies suggesting minimal effect on failure load, stiffness, or displacement at failure and others suggesting up to a 20% reduction in stiffness. BioCleanse® processing and up to eight freeze-thaw cycles (to −80° C) appear to have minimal effect on the biomechanical properties of allografts. Graft preparation, including increasing graft diameter and central location selected within the patellar tendon, were found to improve ultimate biomechanical properties. Grafts from donors younger than 40 years of age are favored to grafts from older patients because they are associated with higher tensile strength and ultimate stress. Finally, there is promise that biologic adjuncts may offer a method for improving the function of allograft tissue, although further research is needed to explore these treatments.

The limitations of this study are related to the limitations of the studies included in the review. The studies in this review were all either time zero biomechanical studies or animal studies and, as such, the study is subject to the same limitations of all time zero biomechanical studies and animal studies. Time zero biomechanical studies most directly simulate the immediate postoperative period and do not consider fixation methods. Additionally, grafts in the biomechanics laboratory are generally subjected to axial loading in tension along the longitudinal axis of the graft and not stresses that mimic conventional in vivo ACL failure modes such as torsional loading. As a result of variability in experimental setup and biomechanical testing parameters evaluated, direct comparison of results among biomechanical studies can be unreliable. Animal studies are limited by differences in time-dependent soft tissue remodeling between animals and humans and animals cannot be subjected to standardized immobilization or physical therapy regimens that may optimize graft incorporation. Because of these limitations, the results from these studies should not influence opinions regarding the rate of graft maturation or contribute to recommendations regarding the appropriate interval for return to sport.

For surgeons using allograft, numerous graft options exist including BPTB, TA, TP, peroneal tendons, quadriceps tendon, hamstring tendons, and iliotibial band/fascia lata. Within the Multicenter ACL Revision Study (MARS) cohort [73], the most popular choice of allograft was BTPB (50%) followed by TA (23%), Achilles tendon (12%), and TP (11%). Biomechanical data presented in this study revealed that among all options, nonlooped tibialis allografts have the lowest LTF and stiffness and that all other grafts demonstrate greater LTF and stiffness than the native ACL. There is limited clinical literature comparing allograft types, although that which is available does not show differences between graft options. Dai et al. [20] demonstrated comparable outcomes between BPTB and hamstring allograft with regard to clinical outcome scores, ROM, Lachman, and single-leg hop test. Kim et al. [40] reported that looped TA and Achilles allografts rendered comparable clinical outcome scores and arthrometric laxity. Based on available biomechanical and clinical data, surgeons should feel comfortable using the allograft of their choice and, when utilized, soft tissue grafts should be should be looped for the strongest biomechanical construct.

The effects of allograft sterilization and preservation have been studied extensively in both the biomechanical and clinical literature. In this systematic review, we determined that moderate-dose irradiation and chemical processing have detrimental effects on biomechanical properties of tissue and should be carefully scrutinized in clinical practice. Studies are mixed on low-dose irradiation (10–12 kGy) with some studies suggesting minimal effect on failure load, stiffness, or displacement at failure and others suggesting up to a 20% reduction in stiffness. BioCleanse® processing and up to eight freeze-thaw cycles (to −80° C) appear to have minimal effect on the biomechanical properties of allografts. Available clinical literature seems to reflect these biomechanical data. Several clinical series reporting results on nonirradiated ACL allografts reported results comparable to autograft tissue with regard to graft failure rates, laxity, and patient-reported outcomes [3, 44]. However, despite favorable clinical results, nonirradiated allografts witnessed increased scrutiny in 2001 after a few highly publicized cases of infection transmission related to musculoskeletal allografts, including a death from *Clostridium sordelli* after an osteochondral allograft and 54 allograft-associated bacterial infections during a 4-year period [12, 13]. As a result, the International Organization for Standardization advocated for routine secondary sterilization to a sterility assurance level of 10^{-6}, a level that can be reached with 9.2 kGy irradiation [4]. The most commonly used methods of secondary sterilization include gamma irradiation and chemical processing. Gamma irradiation of varied intensity may be effective in eradicating different pathogens (5 kGy for nonspore-forming bacteria, 8 kGy for fungi, 21 kGy for bacterial spores, and up to 40 kGy for viruses like HIV and hepatitis C virus), although the clinical role of irradiation remains incompletely defined [68]. Many studies have demonstrated an increased risk of failure, increased laxity by arthrometric testing, and reduced patient-reported outcome scores with allograft tissues treated with chemical processing and irradiation. Sterling et al. [63] reported failure in six of 18 (33%) ACL reconstructions performed with deep-frozen, freeze-dried, ethylene oxide-sterilized BPTB allograft. The authors noted a longer duration of freezing among failed grafts than successful grafts. Rappé et al. [52] demonstrated a dramatically higher failure rate among patients undergoing irradiated (20–25 kGy) Achilles allograft reconstruction than those undergoing nonirradiated Achilles allograft reconstruction (33.3% versus 2.4%). Sun et al. [65] compared 100 patients randomized to BPTB autograft, nonirradiated BPTB allograft, and irradiated (25 kGy) BPTB allograft at a mean of 31 months followup. The authors noted that there was no difference in KT-2000 (MEDmetric, San Diego, CA, USA) laxity between the autograft and nonirradiated groups; however, there was greater laxity in the irradiated group than the two other groups. Although clinical studies using grafts subjected to medium- and high-dose radiation ($\geq$ 20 kGy) have been concerning, clinical results of low-dose irradiated allografts ($<$ 20 kGy) have been more favorable. Ghodadra et al. [27] demonstrated no difference in KT-1000 arthrometric measurements between low-dose irradiated BPTB allograft (10–12 kGy) and BPTB autograft. Chahal et al. [14] similarly reported no difference in personal revision rates between nonirradiated (1.7%) and low-dose irradiated (2.2%) BPTB allograft reconstruction in 477 index reconstructions with BPTB allograft.

Graft preparation, including increasing graft diameter and central location selected within the patellar tendon, was found to improve biomechanical properties. Two biomechanical studies revealed that the central third of the patellar tendon rendered improved maximum load, stress, and stiffness compared with the medial and lateral thirds or with medial or lateral hemipatellar tendons [75, 76]. The authors surmised that this was related to increased thickness at the central third compared with the medial and lateral thirds. The beneficial effect of increasing graft diameter has also been shown clinically in the setting of autograft ACL reconstruction. In a review of 124 hamstring autograft reconstructions, Spragg et al. [62] demonstrated that every 0.5-mm increase in graft diameter (within a range of 7–9 mm) conferred an incremental 0.82 times lower likelihood of graft failure. To optimize the biomechanical strength and to minimize graft failure, every effort should be made to maximize the cross-sectional area of the graft including selection of thicker soft tissue graft, use of

the central third patellar tendon grafts, and the use of looped grafts where possible.

In evaluating donor parameters contributing to ACL allograft properties, we found that donor age plays an important role. Grafts from donors younger than 40 years of age are favored to grafts from older patients because they are associated with higher tensile strength and ultimate stress. In data from the Multicenter Orthopaedics Outcome Network (MOON) group, Kaeding et al. [39] demonstrated a higher rate of retear associated with allograft than autograft reconstruction among patients of all ages; however, failure rates appear to converge around the age of 40 years. Higher rates of graft failure witnessed in young patients may be attributable, at least in part, to the fact that the grafts are from older donors and harbor inferior biomechanical properties to their younger, native tissue. Based on available clinical data, we would recommend caution in use of allografts in patients younger than 30 years. If allografts must be used in younger patients in the setting of revision or strong patient preference, surgeons should make a special request for a donor younger than 40 years of age.

The use of adjuvants such as growth factors and other biologic agents to optimize the postoperative healing environment has recently garnered increased interest in the treatment of many orthopaedic injuries. Two animal studies reviewed in this study demonstrate that VEGF-165, TGFβ-1, and sodium hyaluronate may improve graft biomechanical properties as early as 4 weeks and out to 24 weeks after ACL reconstruction. In the clinical setting, platelet-rich plasma (PRP) has been the most broadly evaluated biologic adjuvant. Although many are hopeful that PRP might accelerate the process of graft maturation and integration, a recent systematic review of 23 studies evaluating the effects of PRP and stem cells revealed no benefit in terms of clinical outcome, bone-graft integration, and prevention of bone tunnel enlargement [22]. Future clinical research might evaluate the use of growth factors like VEGF, TGFβ-1, and mesenchymal stem cells to determine if they are able to accelerate return to play, limit the risk of graft failure, and do so in a cost-effective manner.

In conclusion, there are multiple factors that contribute to the biomechanical properties of allograft tissue in the use of ACL reconstruction. Knowledge of these parameters may influence surgeons' selection of one allograft over another and may help surgeons in stipulating specific graft characteristics when ordering grafts from their local tissue bank. To optimize the biomechanical properties of their allografts, surgeons should use looped soft tissue grafts or central third patellar tendon, avoid grafts subjected to radiation doses > 15 kGy, avoid grafts subjected to more than eight freeze-thaw cycles, maximize graft cross-sectional area, acquire grafts from donors < 40 years of age, and consider the use of adjuvants as more clinical data become available. Surgeons must educate themselves on the processing and sterilization procedures used by their tissue bank to improve clinical care with allograft tissue.

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