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Impact of irradiation on load-to-failure in bone-patellar tendon-bone allografts: A systematic review and meta-analysis

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ABSTRACT

Introduction: To evaluate the impact various levels of irradiation have on bone-patellar tendon-bone (BTB) allograft load-to-failure.

Materials and methods: Pubmed, Google Scholar and Embase were searched for studies reporting load-to-failure measurements of BTB allografts following gamma or eBeam irradiation. All systematic reviews, editorials, as well as studies that utilized animal models and/or other graft sources (achilles, hamstring, quadriceps) were excluded. Meta-analysis was performed to compare the impact of low dose (19 ≤ kGy), intermediate (20–49 kGy) and high dose (>50 kGy) gamma and eBeam radiation on load-to-failure.

Results: Twelve studies, containing a total of 429 BTB allografts (159 controls, 270 irradiated), were identified. Load-to-failure of BTB allograft was significantly decreased at intermediate (20–49 kGy) doses of radiation, while low (≤19 kGy) and high (>50 kGy) doses did not significantly change load-to-failure.

Conclusions: Intermediate doses of radiation may negatively impact the biomechanical integrity of BTB allograft *in vitro*. Future studies are required to examine clinical outcomes at varying irradiation levels.

1. Introduction

Optimal graft type for anterior cruciate ligament (ACL) reconstruction remains a highly debated topic. Various tendon allograft and autograft options exist. Autografts result in lower re-tear rates in younger populations. However, allografts have the benefit of no donor site morbidity and may be a good option for older individuals.[1]

BTB allografts, specifically, are a popular option for allografts with the ability for bone to bone healing.[2] However, all allografts confer the theoretical risk of donor-to-recipient disease transmission. To reduce this risk, the Food and Drug Administration (FDA) and the American Association of Tissue Banks (AATB) created guidelines for sterile processing of allografts. These include donor screening, aseptic harvesting, and terminal sterilization. Terminal sterilization is the use of gamma or electron beam (ebeam) radiation to inactivate pathogens within the graft. Although terminal sterilization is effective at inactivating infectious particles and reducing graft immunogenicity[3], numerous studies have reported reductions in tensile strength, load-to-failure, and stiffness with increased elongation following gamma or electron beam irradiation[[2,4]]. This presents the challenge of balancing the risk of disease transmission with the risk of graft failure from reduced biomechanical integrity.

Multiple studies have investigated the biomechanical impact of varying radiation intensities on BTB allografts *in vitro*. Two types of radiation are commonly used for allograft sterilization, gamma radiation and electron beam (E-beam). Gamma radiation is comprised of photons from radioactive decay and has high penetration with a low dose rate. E-beam is comprised of machine-generated high energy electrons and has a high dose rate and low penetration. Gamma irradiation is the most commonly utilized method of sterilization, with typical doses ranging from 10 to 25 kGy.[5] At these doses, most pathogens are inactivated, and the presumed impact on biomechanical properties is minimal.[6] However, it should be noted that some viral pathogens require higher levels of radiation to be inactivated[7], with doses of more than 30 kGy potentially compromising the viability of the graft.[2] Therefore, with increasing assurance in the sterility of the graft, there may be a decrease in the biomechanical integrity.

While prior literature indicates that irradiation can negatively affect allograft biomechanical properties[8], no previous systematic reviews have focused exclusively on BTB allograft irradiation. The purpose of

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this study was to systematically review existing literature on the biomechanical properties of BTB allografts following various doses of irradiation. Additionally, we aimed to perform a meta-analysis to determine effect size of radiation dose on allografts. We hypothesize that there is a dose-dependent negative impact of radiation on tensile strength for BTB allografts.

2. Materials and Methods

2.1. Eligibility criteria

This systematic review was performed using the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) 27-item checklist and was registered with Covidence (Melbourne, Australia). The inclusion criteria were as follows: (1) study population of BTB allografts after gamma or eBeam irradiation, and (2) inclusion of at least 1 measured biomechanical property (such as load-to-failure, elongation, stiffness). We restricted the articles to those published in full and written in English. The exclusion criteria consisted of systematic or literature reviews, meta-analysis, editorials, and studies utilizing animal models and/or other graft sources such as achilles, hamstring or quadriceps.

2.2. Data sources

PROSPERO, MEDLINE (through PubMed), Google Scholar, and Embase were queried for qualified publications. The searches for qualified literature were performed in May 2022.

2.3. Search terminology

Terms used to search PubMed were: [(bone tendon bone) OR (BTB) OR (patellar-bone tendon-bone) OR (BPTB)] AND [(radiation) OR (irradiation) OR (gamma) OR (ebeam) OR (electron beam)] AND [(Biomechanical) OR (cadaver)] NOT [(Systematic review) OR (Commentary) OR (literature review) OR (meta-analysis) OR (Editorial) OR (porcine) OR (sheep) OR (rabbit) OR (Canine) OR (goat) OR (achilles)]. Google Scholar, and Embase were searched with the terms "(bone tendon bone) AND (radiation OR irradiation) AND (gamma OR ebeam OR electron beam)". PROSPERO was searched with the terms "ACL" AND "allograft" AND "irradiation".

2.4. Study selection

Titles and abstracts were reviewed by two authors (K.M.C. and S.S) who independently reviewed the relevant articles that were extracted for the study. Review authors reached consensus for final data extraction by discussion and extracted the study characteristics. During the initial screening of the extracted articles, articles could only be included or excluded by a unanimous decision from the reviewing authors. After the initial screening, the remaining articles were subsequently reviewed and confirmed by all authors. Data on sample preparation, storage, and testing conditions as well as biomechanical measurements were entered into a database and incorporated into the study accordingly.

2.5. Meta-analysis

Demographic data were extracted and reported as pooled means, when possible. The meta-analysis was conducted using R statistical software (version 4.2.0) to calculate odds ratios (ORs), 95 % confidence intervals (CIs), and mean differences (MDs). A random-effects model was used to pool individual MDs and ORs. Between-trial heterogeneity was determined by performing the I^2 test, with >50 % considered highly heterogeneous.

3. Results

Initial search from the databases Pubmed, Google Scholar and Embase yielded 1639 publications. Removal of duplicates lead to a count of 846. Exclusion of review articles, animal models, clinical studies, studies evaluating non-bone-tendon-bone allografts, and cadaveric studies without a control group yielded 15 papers. Three additional articles were excluded for the following reasons, respectively: no irradiation treatment group, unrelated article, and unrelated article written in French, yielding 12 articles for analysis (Fig. 1).

Allografts were prepared fresh frozen at −20 °C[2,9,10], −50 °C[10], −70 °C[11–13] or preserved using glycerolization and lyophilization techniques[2,14]. Allograft radiation dosages ranged from 7 kGy to 100 kGy of gamma[2,4,9,10,15,16], Ebeam[13,14,17], or both types of radiation (Table 1). One study evaluated 10–12 kGy[4], 1 study evaluated 15 kGy[11], 4 studies evaluated 20 kGy[2,10,15,16], 5 studies evaluated 25 kGy[10,11,13,14,17], 1 study evaluated 30 kGy[15], 5 studies evaluated 34–35 kGy[11–14,17], 3 studies evaluated 50 kGy[14,15,17], 2 studies evaluated 100 kGy[14,17] (Table 1).

The most frequently investigated biomechanical property of irradiated BTB allograft was load-to-failure, discussed in 11 studies[2,4,9–11,13–15,17,18], of which 8 report exact values[4,9–13,15,18]. Other properties frequently reported include stiffness[4,9,11–15,18], strain[4,11–13,15,16,18], elongation[2,4,10,15,18], stress[4,15,16,18] and cyclic elongation[4,12,13,18].

Irradiated BTB grafts tended to have lower load-to-failure compared to non-irradiated grafts. Intermediate (20–49 kGy) doses of radiation significantly ($p < 0.05$) reduced load-to-failure of BTB grafts (Fig. 2). Low (≤19 kGy) and high (>50 kGy) doses of radiation did not significantly decrease the load-to-failure (Fig. 2).

4. Discussion

The most important finding of this systematic review was that intermediate (20–49 kGy) doses of radiation significantly decreased the load-to-failure of BTB grafts, while low (≤19 kGy) and high (>50 kGy) doses did not have a significant impact on load-to-failure.

Previous studies have examined the impact of irradiation on various allograft types. One previous systematic review investigated failure rates of irradiated vs. non-irradiated ACL allografts including achilles, tibialis anterior, BTB, and hamstring, finding no difference in failure between the irradiated and non-irradiated grafts. However, they did find a significant increase in graft failure rates after high (20–25 kGy) vs low (12–18 kGy) dose radiation [6] Another systematic review, found similar results, with increased tissue laxity and higher failure rates at radiation doses of greater than the standard dose of 25 kGy[19]

Many prior studies by Hoburg and colleagues had evaluated the impact of varying levels of irradiation on BTB allografts. These studies found decreases in all tested biomechanical properties at higher doses (34–40 kGy). They also found increased elongation and decreased stiffness, max force, and strain in moderate doses from 20 to 30 kGy[11–13]. However, several of these study conditions and sample preparations varied between publications.

Much of the literature regarding lower levels of radiation (≤19 kGy) was conducted in the 1990s and early 2000s. These studies vary regarding the magnitude of the detrimental effect of low dose radiation on graft biomechanical properties. Our systematic review found that low levels (≤19 kGy) of radiation did not impact load-to-failure testing of BTB grafts. This agrees with prior literature by Yanke et al. which also found no decrease in load-to-failure following gamma irradiation with 10–12 kGy.[4] Hoburg et al. also showed similar results with 15 kGy of Ebeam radiation, finding no significant impact on all tested graft properties, including load-to-failure[13]. Elenes et al. looked at Ebeam and gamma radiation at low and intermediate levels of radiation, also finding no impact on graft properties[18]. In contrast, studies of low dose radiation of other soft tissue ACL grafts, such as hamstring[20] and

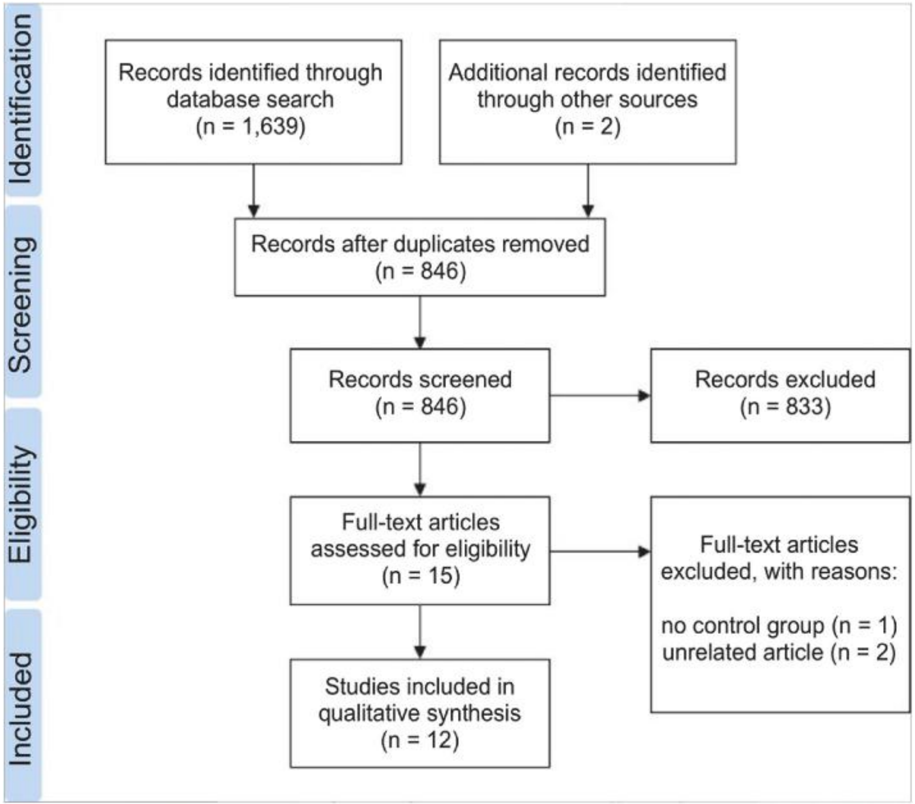

Fig. 1. Prisma diagram.

achilles[21], have found significant but varying magnitudes of decreased mechanical properties with low dose (≤19 kGy) irradiation. At these low doses, Greaves et al. suggests that there is a linear relationship between allograft cross sectional thickness and resistance to degradation of mechanical integrity[22]. This evidence supports our findings that, at low doses (≤19 kGy), BTB allografts maintain integrity.

As mentioned, our study found significantly decreased load-to-failure in the 20–49 kGy group. Interestingly, these results were only observed with grafts preserved by glycerolization or lyophilization[2,14]. This suggests that preservation techniques may also play a significant role in the degradation of allograft biomechanical properties. Compared to fresh frozen graft preparations, glycerolization and lyophilization may alter protein structures. Hoburg et al. also looked at varying levels of gamma radiation in patellar allografts and found a significant decrease in the failure load at intermediate levels (34 kGy), but no decreases at lower levels (10, 15, 25 kGy), including 25 kGy, which is classified as low levels in this systematic review[13]. A follow up paper compared the effects of gamma and Ebeam radiation at two different intermediate levels (25 and 34 kGy). They found lower failure loads in the gamma irradiated group compared to ebeam at 25 kGy and lower failure loads with both groups at 34 kGy when compared to controls[11]. In contrast to our findings, Balsy et al., found no difference in load-to-failure of BTB grafts exposed to 24–28.5 kGy of gamma radiation[10]. Overall, the literature shows a significant impact of intermediate level (20–49 kGy) radiation on BTB allografts. There is also evidence that this impact is multifactorial and further investigation into the interplay of preservation techniques and radiation type should be considered.

Surprisingly, doses greater than or equal to 50 kGy, generally considered to be supra-clinical, did not significantly impact load-to-failure. This finding may challenge a linear correlation with irradiation amount and tendon integrity. Kaminski et al. performed an analysis of tensile strength and failure rates in BTB allografts based on preservation type, irradiation amount, and donor age and found no differences between controls and grafts irradiated with 25, 35, 50, or 100 kGy[17]. A second paper by these authors, also found no difference in failure load of fresh frozen in which tissue was radiated by up to 100 kGy[14]. In contrast, Rasmussen et al. found a decrease in failure load following irradiation with 40 kGy.[9] This suggests, that at higher levels of radiation, the tissue no longer changes biomechanically. Notably, radiation at >50 kGy is not used clinically, so this may not be clinically applicable. Given the hypothesis that irradiation affects the tissue in a dose dependent fashion, one would expect increasing doses of radiation to have a greater impact on the tissue. However, based on existing literature, it appears that the tissue reaches a dose ceiling at which increasing doses of radiation no longer have an increasing impact on the tissue. However, the few studies that included such higher doses of irradiation were limited in sample size. Future studies with larger sample sizes are needed for a more definitive explanation of the observed trend. It should be noted that most studies comparing supra-clinical doses of radiation (>50 kGy) generally examine the molecular transformations of highly irradiated allograft, as opposed to biomechanical strength and durability.

An interesting pattern that arose from this systematic review was the importance of sample preparation on the load-to-failure of grafts. This was a finding in several papers, but not the main focus of many papers in this review of literature. Within the intermediate group, sample preparation seems to have a very large effect on load failure strength. Gut et al. found a significant difference between lyophilization, glycerolization, and fresh frozen grafts all subjected to 35 kGy[14]. At intermediate radiation dosage, the fresh frozen group appeared to resist changes impacted by the radiation better than the lyophilized and the glycerolized group, as shown by a significantly greater load-to-failure in the fresh frozen group[14]. At higher levels, Kaminski et al. found that differences in sample preparation do not appear to be significant [17]. In concordance with these findings, the fresh frozen group in Gut et al. showed no decrease in load-to-failure at radiation doses of up to 100 kGy[14]. In summary, while radiation has a significant impact on the biomechanical properties of BTB allografts, there are likely other factors contributing to

Table 1
Study characteristics.

Study	Journal	Patient Age (mean (range))	Percent Male (%)	# of Grafts	Graft Sizes	Radiation Type	Radiation Doses (kGy)	Preparation/Storage	Mechanical Forces Measured
Curran 2004[2]	Am J Sports Med	51 (25–68)	53.8	26	NR	Gamma	20	Frozen (−20 °C)	Load to failure, elongation, failure mode
Balsly 2008[10]	Cell Tissue Banking	38.2 ± 12.6 (18–55) 32.6 ± 12.2 (18–55)	NR	27	Control 1: CSA: 89.5 ± 28.3 mm^2 18 kGy: CSA: 89.7 ± 25.1 mm^2 Control 2: CSA: 81.7 ± 25.2 mm^2 24 kGy: CSA: 103.6 ± 26.8 mm^2	Gamma	18, 24	Deep Frozen (−80 °C)	Load to failure, failure mode, elastic modulus, maximum strength
Hoburg 2011[12]	Knee Surg Sports Traumatol Arthrosc.	62.5 (43–73)	NR	44	W: 10 mm	Gamma, eBeam, fractionated eBeam	34 (all groups)	Deep Frozen (−70 °C)	Load to failure, cyclic elongation, failure mode, stiffness, strain
Rasmussen 1994[9]	Arthroscopy	38 (18–59)	NR	32	Control: L: 58 mm 40 kGy: L: 57 mm	Gamma	40	Frozen (−20 to −30 °C)	Load to failure, failure mode, stiffness, static creep, peak cyclic creep
Hoburg 2010[13]	Am J Sports Med	48 (19–65)	NR	32	W: 10 mm	eBeam	15, 25, 34	Deep frozen (−70 °C)	Load to failure, stiffness, strain, cyclic elongation
Hoburg 2015[11]	Cell Tissue Bank	NR	NR	50	W: 10 mm	Gamma, eBeam	25, 34	Deep frozen (−70 °C)	Load to failure, stiffness, strain, max strain, creep
Haut 1990[16]	J Orthop Res	34.7±8.2 (20–44)	100	24	Control: W: 14.4 ± 0.6 mm, T: 5.3± 0.2 mm 20 kGy: W: 14.9± 1.0, T: 5.2±0.3 mm	Gamma	20	Frozen (−20 °C)	Load to failure, max stress, max strain, elastic modulus
Fideler 1995[15]	Am J Sports Med	"young"	NR	60	W:10 mm	Gamma	20, 30, 40	Deep Frozen (−70 °C)	Load to failure, elongation, failure mode, stiffness, strain, max stress, modulus
Gut 2016[14]	Cell Tissue Bank	37.1 ±13.5 (17–59)	100	50	Control: CSA:52.8 ± mm^2 25 kGy: CSA: 54.8 mm^2 35 kGy: CSA:50.5 mm^2 50 kGy: CSA: 5.0 mm^2 100 kGy: CSA: 45 mm^2	eBeam	25, 35, 50, 100	Deep Frozen (−70 °C), glycerolization, lyophilization	Load to failure, elongation, relative elongation
Kaminski 2009[17]	Cell Tissue Bank	NR (17–84)	100	50	W: 12 mm (central 3rd of tendon)	eBeam	25, 35, 50, 100	Deep Frozen (−70 °C), glycerolization, lyophilization	Load to failure, failure mode, tensile strength
Elenes 2014[18]	JBJS	57.8 (49–72)	60	40	W: <12 mm	eBeam and gamma	9.2–12.2, 17.1–21, 17.1–21	Deep Frozen (−70 °C)	Load to failure, elongation, failure mode, stiffness, max stress, max strain, elastic modulus, cyclic elongation, static creep, peak cyclic creep, max strength
Yanke 2013[4]	Am J Sports Med	46.5 (24–64)	60 %	27	W: 10 mm	Gamma	10–12	Frozen (−20 °C)	Cyclic elongation, strain, maximum load, elongation at maximum load, maximum stress, strain at maximum stress, and linear stiffness

this effect.

4.1. Limitations

This systematic review is not without limitations. This study was limited by the quality of studies included. Included studies were comprised of small sample sizes (n < 10 for most variables examined). Additionally, these results represent laboratory *in vitro* biomechanical properties. This data should not necessarily be extrapolated to patient outcome data. Lastly, while some studies reported cyclical loading

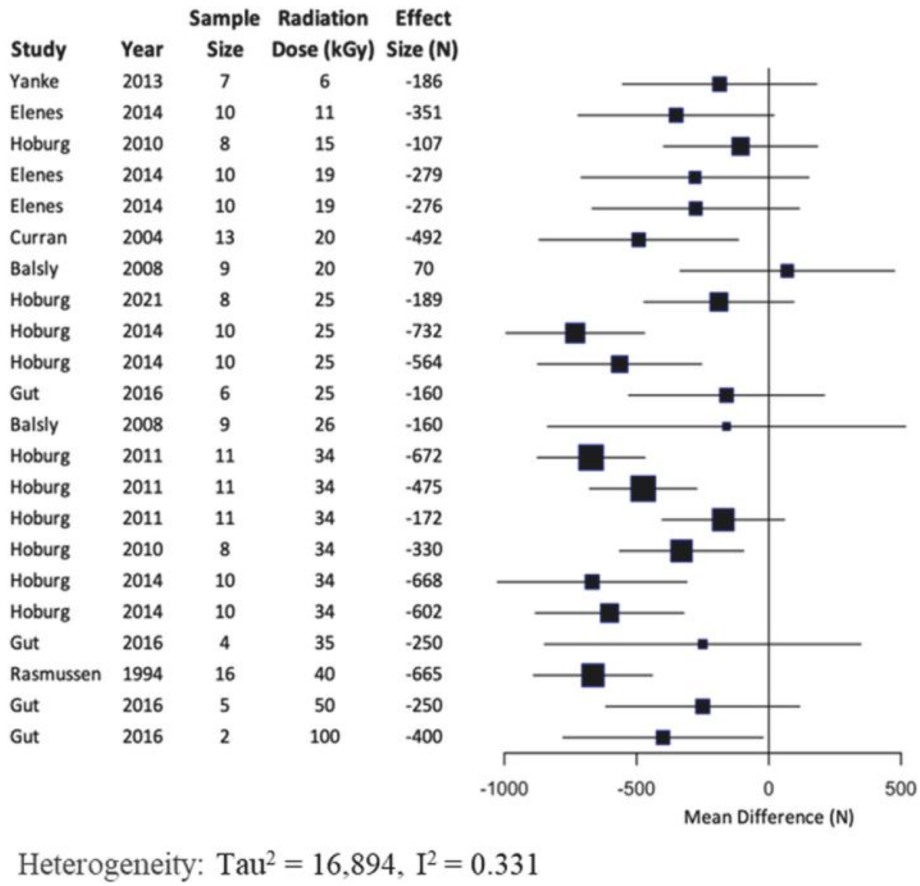

Fig. 2. Difference in load-to-failure of irradiated BTB allograft.

performance, the protocols were varied. Our study primarily examined load-to-failure magnitude. This does not account for repeated cyclic loading of a BTB graft over the lifetime of a patient.

5. Conclusion

Radiation sterilization dose of 20–49 kGy significantly decreased load-to-failure strength of BTB allografts. Graft preparation may also influence biomechanical properties. Further investigation of the effects of graft preparation technique and sterilization will allow for thorough understanding of the biomechanical integrity of irradiated BTB allograft.

Ethical statement

This is not human subject research. The authors agree that this systematic review represents honest and original research.

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Guardian/patient's consent

This is a systematic review and therefore a parent/guardian consent is not applciable

CRediT authorship contribution statement

Kelly M. Chandler: Conceptualization, Investigation, Writing – original draft, Visualization. **Sam Schick:** Investigation, Writing – original draft. **Mathew Hargreaves:** Writing – review & editing, Visualization. **Joseph Elphingstone:** Conceptualization, Methodology. **Eugene Brabston:** Conceptualization, Supervision, Writing – review & editing. **Thomas Evely:** Conceptualization, Supervision, Writing – review & editing. **Aaron Casp:** Conceptualization, Supervision, Writing – review & editing. **Amit M. Momaya:** Conceptualization, Methodology, Writing – review & editing, Project administration.

Declaration of competing interest

The authors have no relevant conflicts of interests to report.

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