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Use of controlled low dose gamma irradiation to sterilize allograft tendons for ACL reconstruction: biomechanical and clinical perspective

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Abstract As reviewed here, numerous biomechanical and clinical studies support the use of controlled, low temperature irradiation of allograft tendons, to provide both excellent clinical results and medical-device grade sterile allografts with minimal risk of disease transmission.

Keywords Gamma irradiation · Bone allograft · Tendon allograft · ACL reconstruction · Allograft safety

Introduction

The preferred method of terminal sterilization for tendon allografts, gamma irradiation, remains a concern to some surgeons. While some older studies have shown that higher, uncontrolled doses of gamma irradiation (>3 Mrad) can have detrimental effects on the strength of allograft tissue, numerous studies suggest that the currently used practices of low dose, controlled, low temperature gamma irradiation are effective to achieve terminal sterilization without detrimental impact on allograft tissue strength. In this review, irradiation methods are presented as well as biomechanical, clinical, and safety assessments of irradiated tendons used for ACL reconstructive procedures.

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Irradiation methods

Prior to assessing studies regarding irradiated tendons, it is important to understand how irradiation methods are reported. Key variables of irradiation include:

- Target dose
- Dose range
- Temperature of irradiation
- Tissue treatment prior to irradiation

Target dose

The targeted dose is that (e.g., 22 kGy) which the tissue is intended to be exposed. However, the manner in which tissue is irradiated in its container or chamber does not allow all tissues to receive an exact similar dose. If only a single targeted dose is reported for tissue treatment, then that is likely the minimal exposure dose. For example, an exposure reported as 25 kGy (2.5 Mrad) likely indicates that all materials received *at least* 25 kGy exposure and that some may have received a much higher dosage (e.g., the outer grafts in a container). Thus, when only a single dose is reported it is fair to consider that to represent the minimal exposure and that some or most tissues will receive a higher dose.

Dose range

The most accurate way to report an irradiation dose is as a dose range, e.g. 15–18 kGy (1.5–1.8 Mrad), which should indicate both the minimum and maximum dose exposure throughout the irradiated container. In order to minimize any negative material impact, it is important that both low doses and tightly controlled dose ranges be employed. It is very difficult to interpret any study that does not include a dose range. Again, if only a single dose is given, it should be considered the minimum exposure.

Temperature of irradiation

The third variable, temperature has been shown to be important as low (dry ice) temperatures serve to minimize any free radical generation and subsequent tissue damage (Anderson et al. 1992; Hamer et al. 1999). Prior to knowledge of the importance of a controlled irradiation dose range at low temperatures, it is difficult to rely on the findings of studies where the details are not reported. Ideally a low average dose, at a narrow dose range, and at low temperatures are all factors in minimizing any irradiation-mediated alteration of material properties.

Tissue treatment prior to irradiation

In addition, the treatment of any tendon prior to irradiation could play a role in how the tissue is potentially impacted. There are certain cleaning and disinfection methods that involve harsh chemicals or physical forces on the tissue. These may damage grafts independent of irradiation or pre-dispose grafts to alteration by irradiation.

Summary

To best interpret study results involving irradiated tissue, it is important to know how a tissue was treated prior to irradiation and exactly how it was irradiated.

Biomechanical assessment of irradiated tendons

In testing biomechanical properties of irradiated tendons, Balsly et al. (2008) reported no change in graft strength or elastic modulus for bone-patellar tendon-bone (BPTB) grafts, anterior tibialis tendons, semitendinosus tendons, or fascia lata soft tissue grafts when treated with sterilizing, low dose (18.3–21.8 kGy) gamma irradiation at dry ice temperatures. Likewise, Greaves et al. (2008) investigated the biomechanical properties of low dose (14.6–18.0 kGy) gamma irradiation on tibialis tendon allografts. In this matched pair study, 63 tibialis tendons were irradiated on dry ice while the contralateral tendons from the same respective donors were not irradiated. The study found that low dose irradiation did not significantly affect the failure load of either single stranded or double stranded tibialis tendon grafts (Table 1).

In a similar study, Roche et al. (2005) investigated the ultimate tensile strength of low dose gamma irradiation (15.4–15.5 kGy) on patellar ligaments and fascia lata allografts. Each irradiated allograft was matched with a control non-irradiated graft from the same donor to limit biomechanical variability resulting from different donors. The study did not find a statistical difference in the tensile strength between the matched low dose irradiated and non-irradiated allografts. In addition, Gibbons et al. (1991) showed in a biomechanical study that maximum stress, maximum strain, and strain energy density to maximum stress was not significantly reduced in goat BPTB grafts irradiated with 20 kGy of gamma irradiation. Further, Goertzen et al. (1995) found no significant difference in strength between canine BPTB grafts irradiated with 25 kGy and non-irradiated BTPB grafts after being implanted in an ACL reconstruction for

Table 1 Failure load (*N*) of non-irradiated and irradiated single-stranded and double-stranded tibialis tendon grafts as reported by Greaves et al. (2008)

	Irradiated	Non-Irradiated
Group 1		
Single stranded	3,062 ± 699	2,843 ± 694
Double stranded	5,124 ± 1,206	5,074 ± 1,032
Group 2		
Single stranded	2,729 ± 995	2,823 ± 573
Double stranded	5,262 ± 845	5,255 ± 706
Group 3		
Single stranded	3,004 ± 603	2,988 ± 787
Double stranded	5,334 ± 1,353	4,971 ± 1,980

No significant differences were found with either treatment. Groups 1, 2, and 3 refer to the donor age of that group (20–45, 46–55, 56–65 years respectively)

12 months. Finally, McGilvray et al. (2005) determined there was no significant difference in the stiffness, ultimate load, and ultimate strength of porcine BPTB grafts treated with 15 kGy versus non-irradiated porcine BPTB grafts. These and other studies (Haut and Powlison 1990; Mae et al. 2003; Maeda et al. 1993, 1998; Smith et al. 1996) of irradiated tendons indicate that treatment below 20–25 kGy have minimal impact on biomechanical properties.

Clinical assessment of irradiated tendons

There is also clinical evidence supporting the utility of low-dose irradiated tendons, which also have the advantage of minimizing any risk of disease transmission. Fanelli et al. (1996) compared irradiated BPTB and Achilles tendon allografts versus BPTB autografts for ACL reconstruction in patients who had combined ACL/PCL instability. No irradiation levels were given, so the presumption is of at least a 15–25 kGy dose. Although the sample size was low (20 patients), the prospective study was the largest study to date (1996) that evaluated ACL/PCL instability. This clinical study found equivalent results between irradiated allografts and autograft tendons. In a technique article, Harner and Elkousy, along with lead author Sekiya et al. (2002), noted they only use patellar tendon allografts that have been irradiated for ACL reconstruction.

In further support, Rihn et al. (2006) investigated the irradiation variable for ACL reconstruction in a clinical study involving 102 patients with an average follow-up of 4.2 years. The study found that 2.5 Mrad of gamma irradiation on BPTB allografts, which is effective in eliminating bacteria, does not compromise the clinical effectiveness of the grafts (Table 2). The authors concluded "[t]hese data suggest that irradiation can be used to sterilize BPTB allograft without adversely affecting clinical outcome."

Taken together, the above articles suggest that a sterilizing dose of irradiation may not be of clinically significant concern when using allografts for ACL replacement. While three reports in particular have presented higher failure rates for irradiated allografts in ACL reconstructions (Rappe et al. 2007; Sun et al. 2009; Prodromos et al. 2007), there are significant questions regarding these studies or data analysis.

Table 2 ACL reconstruction results at average 4.2 years follow-up from Rihn et al. (2006) study using either irradiated BPTB allografts or BPTB autografts

	Irradiated Allograft reconstruction ($n = 39$)	Autograft reconstruction ($n = 63$)	P value
IKDC subjective knee form	86.7 ± 15.5	88.0 ± 13.3	0.65
ADLS[a]	93.4 ± 10.2	92.7 ± 10.5	0.72
SAS[b]	90.1 ± 17.1	90.1 ± 12.8	0.99
Avg 30 lb. KT-1000[c]	1.1 ± 2.5	1.9 ± 2.3	0.11
Avg maximum manual KT-1000[c]	1.3 ± 2.3	2.2 ± 2.0	0.04

[a] Activites of daily living scale

[b] Sports activity scale

[c] Side-to-side difference of anterior translation in mm

Those study limitations are detailed here. The most significant issues with the first study (Rappe et al. 2007) include questionable follow-up methodology and a lack of information regarding the irradiation process used. The study followed up with ~73% of irradiated graft patients compared with ~93% of non-irradiated graft patients. It is unclear why a substantially fewer number of patients in the irradiated graft group returned for follow up care especially since they reportedly had significant graft failure (33%). Also, the method used to irradiate the grafts is unknown, e.g., the temperature of irradiation. The irradiation dose is given as 20–25 kGy and it is unknown if this is a true dose range and what the dose range of irradiation exposure actually was. In addition, other processing methods are unknown, including whether these grafts were also exposed to harsh solvents or cyclic pressures as performed by some banks, such that they may have been prone to damage from the higher irradiation dose used. Also, one surgeon reported twice the failure rate as the other in the study and the recipient age in relation to failures in both groups was not reported, making interpretation difficult. Finally, many tissue providers will routinely treat 'non-irradiated' or 'non-sterilized' grafts with 10–15 kGy doses of pre-processing irradiation for safety reasons so it remains unknown whether the control grafts were truly non-irradiated and also whether the irradiated grafts were double-dosed.

There are also numerous concerns with the Sun et al. study (2009). Only the target dosage is given (25 kGy) with unknown dose range. Also, these grafts were irradiated at room temperature and the grafts were not disinfected prior to irradiation (author, private communication). In addition, grafts were soaked in iodine prior to use. These graft treatments compromise any meaningful interpretation of the results. As significantly, the study has inconsistent results that possibly indicate an issue in either the surgery or measuring techniques. Unfortunately, the allograft patients exhibited a significant increase in duration of post-operative fever over autograft patients. The average duration of fever for an irradiated allograft patient was over 1 week (8.8 days) versus 4.7 days for the autograft patients. In the discussion, the authors state that this high fever rate "was associated with…[different possibilities, or]…the real ability of tissue banks in our country [China] to process allografts". The study also concludes that irradiated bone patellar-tendon bone (BPTB) allografts are clinically inferior to both non-irradiated BPTB allografts and BPTB autografts because of the laxity measurements with a KT-2000 arthrometer. The study reports only 31.3% of irradiated BPTB allografts had less than 3 mm of laxity while 85.3% of the non-irradiated and 87.8% of the autograft group had less than 3 mm of laxity. This is a surprising difference made even larger by the report of 34.4% of the irradiated group exhibiting more than 5 mm of laxity (defined as graft failure by the authors). These extreme percentages should indicate noteworthy irradiated graft patient dissatisfaction as well as significantly different results in other subjective and objective tests. In contrast, however, there were no significant differences in the overall IKDC scores between any of the three groups. Irrgang et al. (1998) noted that the IKDC was an especially rigorous evaluation tool because the lowest score received in any given area becomes the overall score instead of combining the averages like other evaluation systems. This makes the validity of the Sun et al. study even more uncertain since laxity measurement is a component of the overall IKDC score. It is unclear how there was no significant difference in the overall IKDC score among the 3 groups when the irradiated graft group had such extreme laxity measurements. Furthermore, the objective range of motion (ROM), vertical jump, and one-leg hop tests found no significant difference in any of the groups. There were also no significant differences among the 3 groups for mean Lysholm, Tegner, or Cincinatti knee scores. Moreover, there was not a significant difference in patients' satisfaction with their ability to participate in sports in any of the groups. One would expect significant differences in all or most of these tests and responses if 68.7% of irradiated allograft patients had greater than 3 mm laxity. The IKDC system is one of the best evaluation tools to measure ACL reconstruction results (Foster et al. 2010) and the results of this test and all the others should have been balanced against the arthrometer measurements. This balance is particularly important because there may be no correlation between laxity measurements and functional outcome (Mirzayan 2005). Pollet et al. (2005) investigated this correlation in a prospective, clinical study of 29 ACL deficient patients with an average 33 month followup. After comparing anterior knee laxity, questionnaire based on IKDC score, sports activity rating scale (SARS), activities of daily living (ADL), and other tests, the study found "no correlation between the joint laxity and the functional outcome score." This lack of correlation is actually supported by the Sun et al. (2009) study in which, according to the authors, almost 4 times as many irradiated allograft patients had graft failure based on laxity measurements but there was no statistical difference in patients' satisfaction in their postoperative sports activity or overall IKDC score. At the very least, the inconsistent results and non-standard tissue treatment methods should have given the authors pause before making the recommendation to completely discontinue use of irradiation to sterilize allografts.

Prodromos et al. (2007) performed a meta-analysis on stability of autografts and allografts for ACL reconstruction. This included a sub-analysis of non-irradiated vs. irradiated grafts. They came to the following conclusion: "The direct deleterious effects of graft radiation are an additional area of concern. The stability rate in the radiation-sterilized grafts in this study was strikingly low." However, the data used to draw this conclusion is heavily skewed by one particular study. In detail, to examine irradiated tendons, the authors included three studies, here called Noyes, Gorschewsky, and Rihn. They based conclusions on normal stability rate (which was 43% for irradiated vs. 63% for non-irradiated allograft) and

abnormal stability rates (which was 31% for irradiated and 12% for non-irradiated allografts). This certainly appears negative for irradiated allografts. However, the irradiated group included in the Gorschewsky study included more grafts, and thus was more heavily weighted, than the other two studies combined and, most importantly, included a process method with steps containing *acetone, sodium hydroxide,* and *hydrogen peroxide*. These harsh chemicals can be quite damaging to soft tissues and no conclusion can be drawn from the fact that these grafts were also irradiated unless the proper controls were included (treatment with these chemicals without irradiation). If this single study is removed from the meta-analysis, then the comparisons become: normal stability rates of 62% for irradiated vs. 63% for non-irradiated allografts and abnormal stability rates of 15% for irradiated and 12% for non-irradiated allografts, respectively. Thus, the exclusion of the harsh chemically treated graft data set, yields results suggesting equivalent performance for irradiated vs. non-irradiated grafts. Further, note that this study that was, in fact positive regarding irradiation reported on tissue irradiated with 2.5 Mrad, which is even above commonly used levels of 13–18 kGy, further suggesting the utility of terminal sterilization via gamma irradiation.

Irradiated tissue safety

It appears that there still exists confusion as to the definitions of sterility and processing methods. Sterile or aseptic tissue recovery by itself is mistakenly considered as a method that will result in the supply of sterile grafts hence making terminal sterilization unnecessary (Marrale et al. 2007). Recovery under aseptic conditions seeks to ensure that no further bioburden is introduced from the environment but does not remove existing bioburden in the tissue (Vangsness 2004; Vangsness et al. 2003). Tissue banks must use disinfection steps and/or terminal sterilization to accomplish sterilization of existing bioburden. Furthermore, aseptic recovery occurring in a surgical operating room can only result in, at best, a sterility assurance level (SAL) of 10^{-3}, and then only if properly validated, versus a terminal sterilization SAL of 10^{-6}. Sterility assurance level gives the probability of there being viable microorganisms on a particular graft unit, instrument, etc. A SAL of 10^{-6} indicates there is only at most a 1 out of 1,000,000 chance that a viable organism exists with any single graft compared with an SAL of 10^{-3} which indicates a 1 out of 1,000 chance (Vangsness et al. 2003). Some tissue banks choose to use terminal sterilization to increase the likelihood of the safety of their tissue. If the allograft can be guaranteed to a SAL of 10^{-6} then it may possibly possess an even lower degree of infection risk than an autograft procedure (Bryans et al. 2010; Katz et al. 2008).

While the potential risk of viral transmission is extremely serious, it should be kept in perspective that this risk is virtually non-existent if the allograft is procured from a bank using intensive donor screening, tissue disinfection procedures, and terminal sterilization methods that inactivate viruses. While some studies reported that at least 30 kGy of gamma irradiation is needed to inactivate HIV, these studies have assumed HIV is present in high density levels (Fideler et al. 1994; Hernigou et al. 2000). At least 30 kGy may be necessary to inactivate high density amounts of HIV but is excessive for lower density levels of the virus. If in the extremely unlikely event that HIV is present at all, the donor screening and tissue disinfection procedures help ensure that the virus will only be present in extremely low density amounts. The low 10–20 kGy dosage used for terminal sterilization is able to deactivate 99.9% of any remaining low-density HIV in allograft tissue (Moore 2010).

Discussion

The preferred method of terminal sterilization for allografts, gamma irradiation, remains a concern to some surgeons. However, while some studies have shown high dose gamma irradiation (>3 Mrad) can have detrimental effects on the strength of allograft tissue, numerous studies have shown that the currently used controlled and low doses of gamma irradiation are effective in terminal sterilization and have no detrimental effect on allograft tissue strength. Rihn et al. (2006) determined that not only is using 25 kGy of gamma irradiation on BPTB allografts effective in preventing bacterial infection but it does not compromise the clinical effectiveness of the graft. The results are also comparable for soft tissue allografts. Balsly

et al. (2008) reported no change in graft strength for patellar tendons, anterior tibialis tendons, semitendinosus tendons, and fascia lata soft tissue grafts when low dose gamma irradiation was used to terminally sterilize at low temperatures. Greaves et al. (2008) found low dose gamma irradiation did not affect the strength or stiffness of soft tissue tibialis tendon allografts. The terminal gamma irradiation is necessary in order to provide allograft tissue with a SAL of 10^{-6} which is equivalent with implantable medical devices. Low dose gamma irradiation (10–15 kGy) in combination with donor screening and tissue processing procedures allows for thorough bactericidal treatment while maintaining intrinsic biomechanical properties and ensuring successful clinical performance (Block 2006).

Conclusions

As reviewed here, numerous biomechanical and clinical studies support the use of controlled, low temperature irradiation of allograft tendons, to provide both excellent clinical results and medical-device grade sterile allografts with minimal risk of disease transmission.

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