Low-Dose Irradiation of Allografts for Ligament and Tendon Reconstruction

Arthrex Research and Development

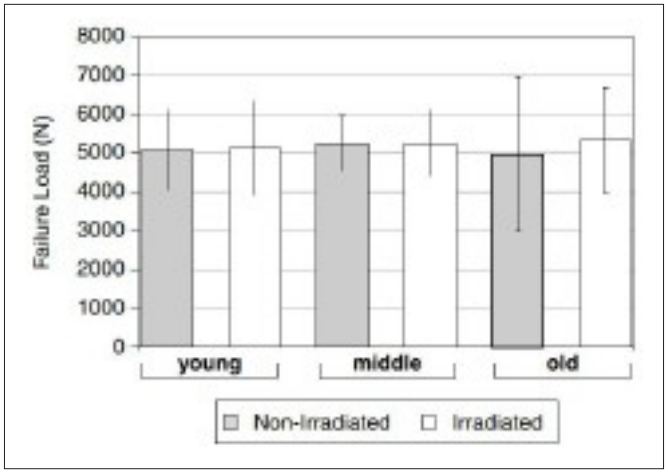
Introduction

Soft tissue allografts for ligament and tendon reconstruction have been used successfully for many years, and their usage has increased dramatically in the past decade. The rate of soft tissue allograft usage in primary anterior cruciate ligament (ACL) reconstruction vs. autograft usage went up from 17% of all surgeries in 2002 to 46% of all surgeries in 2008[1]. Allografts are either processed aseptically, with a sterility assurance level (SAL) of 10-3 if properly validated, or terminally sterilized at a SAL of 10-6. Low-dose, low-temperature gamma irradiation as a terminal sterilization method to treat aseptically processed tissue was introduced to provide a safe and reliable allograft option for surgeons and their patients, due to the previous history of allograft recalls. From 1994 to 2007, almost all allograft recalls were for musculoskeletal tissues, and they were due to improper or incomplete donor evaluation, contamination, recipient infection, and positive serologic tests[2]. Even with increased regulation by the American Association of Tissue Banks (AATB), allograft recalls can still occur. Therefore, there is an increasing interest in sterilization methods that yield a SAL of 10-6. This white paper will demonstrate that irradiated allografts provide the necessary biomechanical strength and safety needed for ligament and tendon reconstruction.

Human *Ex Vivo* Biomechanical Studies

There have been many human *ex vivo* mechanical studies showing the utility of low-dose irradiated soft tissue allografts. A study by Greaves *et al* looked at single-stranded (SS) and double-stranded (DS) tibialis allografts[3]. All allografts were processed aseptically and treated with the Allowash XG® process from LifeNet Health. Half of the tendons were irradiated at a range of 1.46-1.80 Mrad at dry ice temperatures, while the other half was not irradiated. Besides being separated by strand diameter, they were also separated by age group—ages 20–45 (young), 46–55 (middle), and 56–65 (old). The failure load was similar for the irradiated and non-irradiated grafts in both the SS and DS groups, and it did not change by age group. Figure 1 shows the failure loads for the DS grafts by age group; the failure loads for the SS grafts by age group were not graphed.

Figure 1:



Failure load of irradiated and non-irradiated DS tibialis allografts sorted by age (from Reference 3)

A 2008 study by Balsly *et al* took a look at the biomechanical properties of bone-patellar-bone (BTB), anterior tibialis tendons, semitendinosus tendons, and fascia lata grafts[4]. The grafts were processed aseptically, treated with the LifeNet Health Allowash XG® process, and sterilized with low-dose (1.8-2.2 Mrad) gamma irradiation at dry ice temperatures. Note that this testing was done at a higher than normal irradiation dose, compared to the normal dose of less than 2.0 Mrad, to test worst-case conditions. The study concluded that there was no change in tensile strength or elastic modulus for the different types of grafts tested at low doses compared to matched control aseptically processed, non-irradiated grafts.

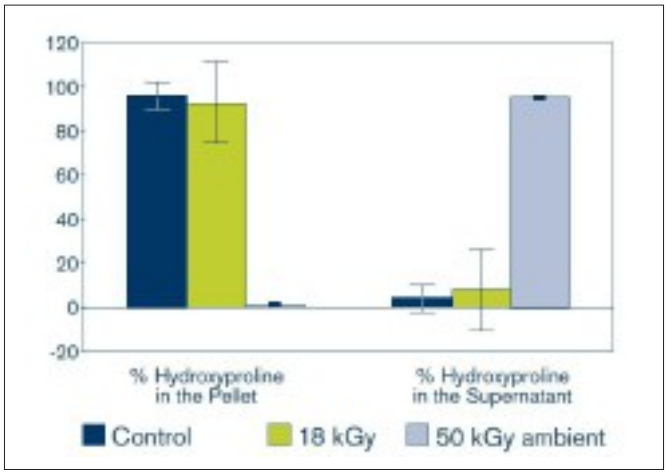
Yanke *et al* investigated the use of irradiated BTB allografts[5]. The allografts were aseptically processed and treated with the AlloTrue™ process from AlloSource. For the 10 sets of matched pairs used in the study, one graft from each matched pair was irradiated at a low-dose range of 1.0–1.2 Mrad, while the other graft was not irradiated. Although not mentioned, the AlloTrue™ process delivers low-temperature irradiation[6]. The maximum load during cyclic testing was not affected by irradiation. Maximum stress, elongation, and strain were also unchanged due to irradiation. Overall, the study concluded that there were no differences between irradiated and non-irradiated grafts.

Conversely, high doses of irradiation can lead to decreased mechanical properties of the soft tissue allografts. In an article from Fideler *et al*[7], four groups of BTB allografts were tested to failure—fresh frozen non-irradiated control grafts and fresh frozen grafts irradiated at 2.0, 3.0, and 4.0 Mrad doses at dry ice temperatures to avoid sample thawing. The stiffness and maximum elongation of the 2.0 Mrad samples were not significantly different from the non-irradiated control. However, as the irradiation dose rose from 2.0 to 4.0 Mrad, there were significant drops in stiffness and maximum elongation at each increasing dose. Even though the paper did not characterize the processing and treatment techniques prior to irradiation, it is apparent that an increase in irradiation dose at dry ice temperatures leads to decreased mechanical properties.

An article from Rasmussen *et al*[8] showed that BTB grafts irradiated at 4.0 Mrad at dry ice temperatures (as defined in a previously published paper[9]) compared to non-irradiated matched pair grafts had lower stiffness and maximum load. However, there were no differences in static or cyclic creep. Even though it is unclear how these results would relate to clinical parameters, it is clear that the high 4.0 Mrad dosage definitely affected tendon mechanical properties. The allografts were obtained from different locations, but were likely all processed in a similar manner prior to irradiation.

The lack of a mechanical difference between non-irradiated tendons and low-dose, low-temperature irradiated tendons can be explained by the absence of tissue collagen breakdown within the irradiated samples. This was demonstrated using a chymotrypsin sensitivity test on the allografts. Irradiated and non-irradiated samples were ground down, then the enzyme chymotrypsin was added to break down damaged (denatured

Figure 2:



The effects of irradiation at dry ice temperatures on collagen breakdown in tibialis tendon allografts (from Reference 10)

and fragmented) collagen within each sample. The entire sample is then centrifuged, which creates a solid pellet on the bottom and a liquid supernatant on the top. Both are measured for the amount of hydroxyproline, an amino acid in collagen. Ideally, there should be a high percentage of hydroxyproline in the pellet (normal, non-damaged collagen) and a low percentage in the supernatant (fragmented, denatured collagen). This indicates that the sample did not have significant collagen breakdown. Using this test, non-irradiated and 1.8 Mrad-irradiated Allowash XG®-treated tibialis tendons demonstrated similar high percentages of hydroxyproline in the pellet and low percentages in the supernatant, indicating the tendon collagen fibers were not altered. However, 5 Mrad-irradiated tendons had a low percentage of hydroxyproline in the pellet and a high percentage in the supernatant, indicating there was collagen breakdown due to the high dose, ambient temperature irradiation (Figure 2)[10,11]. Similar results have also been demonstrated in allograft bone[12]. Therefore, it is apparent that low levels of irradiation at 2.0 Mrad or less at dry ice temperatures do not cause significant collagen breakdown of the soft tissue allograft.

Human Clinical Studies

Of course, the most important question to clinicians and patients is if soft tissue allograft irradiation leads to decreased clinical outcomes.

One study looked at the clinical outcomes of patients implanted with BTB allografts irradiated at a dose of 2.5 Mrad (n = 39) vs. BTB autografts (n = 63) at a 4-year follow-up[13]. The allografts were obtained from a single unnamed source and irradiated at a single location, but the irradation temperature was not mentioned. At follow-up, patients implanted with allografts had similar International Knee Documentation Committee (IKDC) Subjective Knee Form scores as those patients implanted with autografts. Other outcome measurements (Activities of Daily Living Scores (ADLS), the Sports Activity Scale (SAS) of the Knee Outcome Survey (KOS), and the Short Form-36 (SF-36)) were also similar for both groups. In addition, different measurements for laxity (maximum manual KT-1000, Lachman, pivot shift, posterior drawer, and varus/valgus stress) were performed and there were no differences. Lastly, the percentage of patients returning to strenuous sports in both groups was similar. This study demonstrated no clinical differences between the performance of irradiated BTB allografts and autografts.

Another study looked at laxity and KT-1000 measurements for patients implanted with BTB autograft (n = 132) and BTB allograft (n = 106) between March 2002 and March 2006, measured from 6 weeks to 1 year follow-up[14]. Even though all allografts were obtained from AlloSource, the allografts implanted after June 2004 (n = 58) were irradiated at a dose of 1.0-1.3 Mrad, while those implanted before June 2004 (n = 48) were not irradiated. Since there was no statistical difference in KT-1000 measurements between 6 weeks to 1-year follow-up for irradiated and non-irradiated allografts ($p > 0.05$), the paper combined all allograft information into one group. There were no differences in laxity or KT-1000 measurements between BTB autografts and BTB allografts, from 6 weeks to 1-year follow-up. This is another study showing that irradiated allograft had similar clinical outcomes to autograft.

A few clinical studies emphasize the importance of keeping a low temperature during irradiation. A study that focused on 6-month follow-up of patients implanted with Achilles tendon allografts showed a significant difference in graft failure rates[15]. The grafts irradiated at a dose of 2.0-2.5 Mrad had 11 out of 33 complete failures, compared to 1 of 42 complete failures in the non-irradiated grafts. All grafts were obtained from the same unnamed tissue bank, where they were aseptically processed, deep frozen, and stored at low temperatures. Unfortunately, the irradiation temperature was not defined. If the grafts were irradiated at room temperature, it is likely that free radical formation damaged the grafts, which could cause the negative results seen with the irradiated grafts[16]. However, if the grafts were irradiated at low temperature, free radical formation is minimized. Two other studies comparing BTB irradiated allografts to BTB autografts and BTB aspetically-processed allografts had similar poor clinical findings for irradiated allografts[17,18]. The irradiation dose was no higher than 2.5 Mrad, but the irradiation temperature again was not defined. Therefore, it is important that if poor clinical results are observed with

low-dose gamma irradiation, the temperature of the irradiation process needs to be noted. In addition, it is important to understand what processing technique was used for cleaning. For example, when acetone baths are used, this can lead to allograft disruptions, as described below.

From all of the positive and negative papers, it is apparent that low dose irradiation at 2.0-2.5 Mrad or less, always performed at a low temperature, does not decrease the clinical response of the soft tissue allograft.

Sterilization Processes

The sterilization methods used by different tissue banks need to be clearly defined, as each process has its own variations between aseptic processing and/or other processses such as chemical processing or irradiation. The major allograft cleaning and sterilization processes[19,20] include the Allowash XG® process from LifeNet Health, the AlloTrue™ process from AlloSource, the Tutoplast and BioCleanse processes from RTI Biologics, the Clearant process, and an aseptic processing protocol from the Musculoskeletal Transplant Foundation (MTF).

The Allowash XG® process from LifeNet Health to clean and terminally sterilize allograft involves[10]:

- bioburden control, involving meticulous and rigorous screening based on FDA and AATB guidelines and strict donor exclusions
- bioburden assessment, involving extensive serologic testing for microbiological contamination, including bacteria, fungi and infectious diseases
- minimized contamination during recovery, including state-of-the-art processing to maintain or further reduce an already low bioburden
- rigorous cleaning, involving flushing, centrifugation, hypotonic processes, and ultrasonication to solubilize and remove blood elements (i.e., marrow and lipids)
- disinfection and rinsing, involving an intensive decontamination, disinfection and scrubbing regimen to remove and eliminate viruses and bacteria, and centrifugation or microabsorption to remove residual water and
- terminal sterilization, involving a low-level dose of gamma irradiation (less than 2.0 Mrad) at low (dry ice) temperatures, with minimal to no effects on mechanical strength[21].

AlloSource uses the AlloTrue™ process to clean and terminally sterilize their allografts, and this involves[6]:

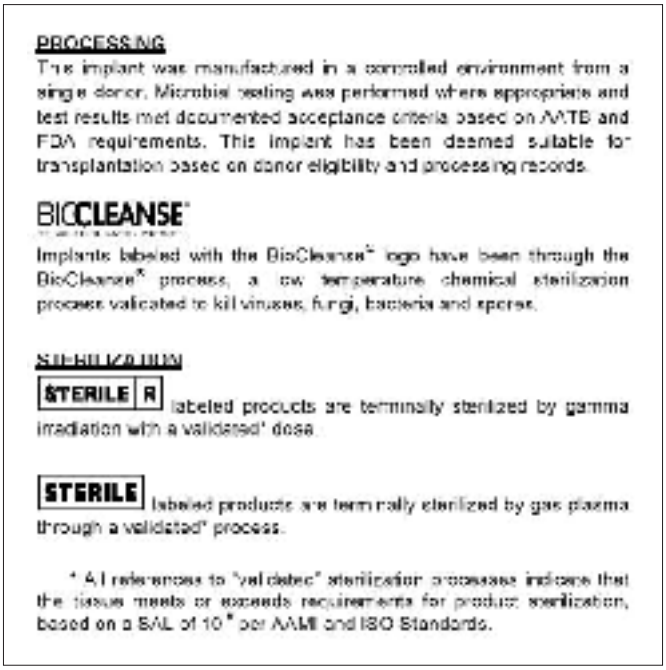
- stringent donation screening procedures involving strict donor criteria, extensive serology testing, medical social history, and physical assessment
- initial tissue processing, including initial physical inspection, rigorous tissue inspection, the use of sterile or USP 24 Grade water and ISO Class 5-7 clean rooms, and continuous environmental monitoring to detect potential contaminants
- preparation for the AlloTrue™ cleaning process, including developing a customized cleaning and reagent administration based on specific tissue type and accurate weight measurement with centrifugation, using airtight ultrasonic containers that prevent overexposure to solutions and temperature and reduce the risk of contamination
- the AlloTrue™ cleaning process, which reduces the bioburden of the processed tissue and involves sonication to loosen blood and lipids, rotation for even and dynamic distribution of reagents and rinses, and temperature control to reduce the risk of tissue damage and
- post-treatment/extraction, including low dose (1-1.3 Mrad), low (dry ice) temperature irradiation that gives the tissue a SAL of 10-6 and full water immersion extraction to measure any residual microbial presence

Some implants from RTI Biologics are processed using the Tutoplast process, and this involves[22]:

- serological screening for bacteria, fungi, and viruses
- lipid removal and viral inactivation using an acetone bath
- bacteria removal using hyperosmotic saline and distilled water baths
- prior inactivation with a sodium hydroxide bath
- protein removal and further viral inactivation with a hydrogen peroxide bath
- a final acetone bath with vacuum extraction for storage and room temperature and
- terminal sterilization with irradiation at a 1.78-2.01 Mrad dose, where the temperature is not mentioned

Processes utilizing acetone baths undergo a severe dehydration process that could compromise the strength of the allografts. This is demonstrated in a paper from 2005[23], where patients that received BTB allografts processed using the Tutoplast process were compared to patients that received BTB autografts at 2 and 6 years post-surgery. Out of 201 patients studied at 2 years, there were 97 BTB allografts and 104 BTB autografts. The allograft group had 20 patients with complete rupture (20.6%), while 5 patients had complete failure in the autograft group (4.8%). Out of 186 patients studied at 6 years, there were 85

Figure 3



PROCESSING

This implant was manufactured in a controlled environment from a single donor. Microbial testing was performed where appropriate and test results met documented acceptance criteria based on AATB and FDA requirements. This implant has been deemed suitable for transplantation based on donor eligibility and processing records.

BIOCLEANSE®

Implants labeled with the BioCleanse® logo have been through the BioCleanse® process, a low temperature chemical sterilization process validated to kill viruses, fungi, bacteria and spores.

STERILIZATION

STERILE R labeled products are terminally sterilized by gamma irradiation with a validated* dose.

STERILE labeled products are terminally sterilized by gas plasma through a validated* process.

* All references to "validated" sterilization processes indicate that the tissue meets or exceeds requirements for product sterilization, based on a SAL of 10^{-6} per AAMI and ISO Standards.

From Wright Medical Technology ALLOPURE Evans and Cotton Allograft Bone Wedge Instructions for Use, RTI document S01169, Version 5763 R1 04/22/10

BTB allografts and 101 BTB autografts. The allograft group had 38 patients with complete rupture (44.7%), while 6 patients had complete failure in the autograft group (5.9%). The paper concluded that using Tutoplast-processed allografts for physically active patients should not be done.

Other implants from RTI Biolgics are processed using the BioCleanse™ process, and this involves[24]:

- removal of blood, lipids, and marrow (in bone) from tissues through a vacuum/pressure process, which reduces risk and recipient immune response;
- chemical sterilants that completely penetrate tissues, and are validated to eliminate pathogenic organisms including HIV, hepatitis B and C, bacteria, fungi, and spores
- removal of germicides, leaving the tissue biocompatible; and
- post-processing testing for sterility confirmation, environmental controls, strength evaluation, and residual moisture

The BioCleanse™ process is promoted as an automated, validated sterilization process to achieve a SAL of 10-6 without terminal sterilization. However, the labeling associated with these soft tissue allografts do not indicate that the grafts are sterile and this is because they do not go through a terminal sterilization (Figure 3). Since the soft tissue allografts are manually packaged, this runs the risk of contaminants being introduced during the packaging process. BioCleanse™ bone grafts, bone paste, and membrane tissue, however, are all exposed to a terminal sterilization process to provide a final product with a SAL of 10-6.

The Clearant Process

The Clearant process freezes the graft, removes the water in the graft, and adds dimethylsulfoxide (DMSO) as a radioprotectant to protect the graft from free radical formation during irradiation. The graft is then irradiated at a dose of 5 Mrad at low temperature. One clinical study was found on patients implanted with Achilles allografts that were either processed aseptically (n = 28) or with the Clearant process (n = 54)[25]. Follow-up for the control group was 31.4 months, while the follow-up for the Clearant process was 19.9 months, and all analyses were done pre-surgery and post-surgery. Even with the difference in follow-up time between groups, The Tegner activity level scores were similar for both groups pre- and post-surgery. Effusion improved substantially as well for both groups. Lastly, range-of-motion was improved by 9° for the control group and 17° for the Clearant group post-surgery.

MTF Process

Lastly, MTF utilizes an aseptic processing protocol on their allografts. However, about 65% of their unprocessed tissue is gamma irradiated at a dose of 1.2-1.8 Mrad at frozen temperatures[26]. MTF claims this irradiation acts as a pre-treatment decontamination step to reduce bioburden prior to aseptic processing, and that it is not classified as terminal sterilization. Recently, however, it has been anecdotally reported that more than 65% of their unprocessed allografts are being gamma irradiated in an attempt to reduce bioburden before processing. MTF recently received a warning letter from the FDA regarding some of their cleaning and aseptic processing procedures[27], but the move to gamma irradiate all specimens is unlikely related to the warning letter.

Other Concerns with Allografts

As described above, if the soft tissue allograft is processed appropriately without solutions that damage the tissue quality, and if a low dose, low temperature irradiation process is utilized for terminal sterilization, the allografts mechanical properties will not be affected. However, because a low dose of irradiation is recommended, this brings up the concern of potential disease transmission. All allograft donors are tested for the human immunodeficiency virus (HIV-1 and HIV-2), hepatitis B and C, human T-lymphotropic virus (HTLV-I and HTLV-II), and syphilis prior to any tissue recovery, as per AATB provisions instituted in 2007[19]. These testing procedures result in a reduced risk of transmission of viruses such as HIV, with the reported rate being 10-6 with current testing procedures[28]. There have been questions as to whether low doses of gamma irradiation are sufficient to kill viruses. This concern has been minimized by a study published in 2012 showing that a dose of 1.16-1.29 Mrad at low temperature is sufficient to inactivate HIV, hepatitis A, porcine parovirus, pseudorabies virus, and bovine viral diarrhea virus[29].

A study published in the Journal of Bone and Joint Surgery found that infection rates in allografts from a single tissue bank (MTF) vs. autografts for ACL reconstruction were not statistically different from each other[26]. The overall rate of infection in the study was 2.32%. A recent abstract from the 2013 meeting of the Arthroscopy Association of North America (AANA) found that hamstring autografts for ACL reconstruction had higher incidences of infection compared to BTB autografts or allografts from different locations (BTB, hamstring, etc.)[30]. A study by Crawford *et al* reviewed surgical site infections after ACL reconstructive surgery from one outpatient surgical center[31]. During the study period, 331 ACL reconstructions were performed, using 290 allograft tendons (250 aseptic/40 sterile) from 8 different companies and 41 autografts. The study concluded that the infection rate for patients who received aseptically processed tissue was 4.4% compared to 0% for sterile allografts and autografts.

Conclusion

This paper references numerous articles that show that the use of irradiated allografts can provide excellent clinical results and the assurance of sterility through low dose irradiation at low temperatures. Many tissue providers now incorporate the use of dry ice temperatures to minimize the effects of free radicals on the collagen bundles within soft tissue allografts. While the chances of disease transmission are extremely rare, by providing a terminally sterilized allograft, the chances of complications and infections arising from the allograft are now reduced even further.

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