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# The Effect of Donor Age and Terminal Sterilization on the Biomechanical Properties of Human Tendon Allografts

LifeNet Health®
BIO-IMPLANTS DIVISION

# The Effect of Donor Age and Terminal Sterilization on the Biomechanical Properties of Human Tendon Allografts

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## Abstract

Human tendon allografts are widely used in reconstructive surgery. Tissue quality and performance can be dependent on a number of factors including characteristics such as donor age and tissue processing conditions. The performance of tissues and patient outcomes may also be compromised if any infectious agent is transplanted along with the allograft. Thus, some tissue banks employ terminal sterilization methods to ensure minimal risk of disease transmission. Here, we review the impact of both donor age and terminal sterilization by gamma irradiation on tissue properties. In summary, under the appropriate tissue selection methods and careful processing, tendon grafts representing donor age up to 70 years old and terminally sterilized by proprietary methods can be appropriate for surgical use.

## Introduction

In the clinical setting, soft tissues are used in the repair of various structures. Of particular focus here, repair or replacement of the anterior cruciate ligament (ACL) has become the most common soft tissue reconstructive procedure in orthopaedic practice. To facilitate this procedure, numerous graft substitutes are in use with the goal of reproducing the complex anatomy of the native ligament or tendon, emulating normal strength and stiffness, allowing strong, secure fixation and subsequent biological incorporation, and minimizing morbidity and post-operative rehabilitation.[1] Autograft tissue has been considered an excellent option for source material and a variety of graft types are used for soft tissue repair.

Operative and postoperative complications (such as weakness of knee flexion and loss of range of motion) that can occur with autologous graft harvesting have been leading surgeons to seek alternatives to autografts.[1,2] Human allograft tissue offers several distinct advantages over autografts including shorter operative times, reduced surgical morbidity, decreased postoperative pain, and improved cosmesis. As a result, musculoskeletal allograft tissue is increasingly being used for orthopaedic applications.

Despite the advantages of allograft tissue noted above, appreciation of the risk of disease transmission and the impact of tissue processing methods are important surgical considerations. To address the risk of disease transmission, many tissue banks have employed terminal sterilization methods using gamma irradiation. While these methods may alleviate the concern of disease transmission, it is accompanied by new concerns regarding the impact of these methods on tissue performance. In addressing this issue, please note the study by Rihn et al[3] in which patients were evaluated who had undergone endoscopic ACL reconstruction with either an autograft or disinfected and irradiated allograft patellar tendons. After an average follow-up time of 4.2 years, patients in both groups had similar clinical outcomes. This study indicates that allografts that have been both disinfected and irradiated to eliminate the risk of disease transmissions are still wholly functional in the clinical setting. This finding is supported by biomechanical studies as reviewed here.

## Biomechanical Properties of Native Anterior and Posterior Cruciate Ligaments

In order to better gauge the requirements of soft tissue grafts used in the repair or reconstruction of the ACL, the biomechanical properties of native ligaments should be reviewed. The strength required for activities of daily living were estimated by Noyes et al[4] to be 454 N based on the failure strength of the ACL. In the authors' words, "it seems reasonable to assume that under normal conditions biological tissues are subject to forces ranging from one-tenth to not more than one-fifth of their breaking loads." More detailed analyses were performed by Morrison[5,6,7] regarding the forces that the ACL and PCL (posterior cruciate ligament) are subjected to during activities of daily living. An overview of these data is shown in Table 1. It should be kept in mind that forces produced during athletic activities could be higher.[8]

The native cruciate ligament isolated from cadaveric donors has also been evaluated. The ultimate load to failure of the native ACL, defined as the force tissue can tolerate before failure, has been studied by numerous investigators over several decades. This value has been reported to range from 658 to 2195 N. Tendon stiffness, which is a measure of the resistance offered to external loads as a specimen deforms, has likewise been demonstrated to range between 129 and 306 N/mm (Table 2). While there is an apparent age-related impact on biomechanical properties of these tissues, note that the lowest ultimate load to failure was 658 N in the age group up to an extended age of 97 was well above the 454 N load required for 'daily living' as reported in the previous paragraph. Also, the grafts that were tested here would not have been prescreened for visual acceptance as a tissue bank would in handling clinical tissues and thus would represent a worst case situation.

**Table 1:** *Estimation of forces on the cruciate ligaments in activities of daily living*

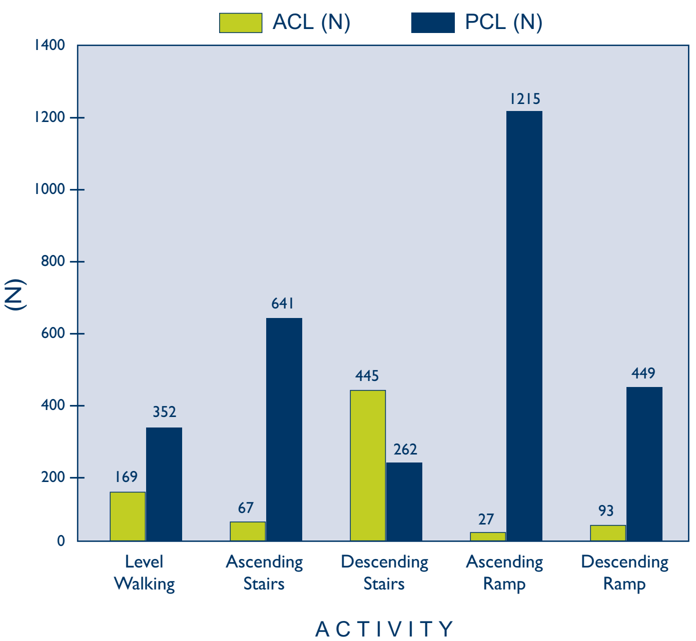



**Table 2:** *Ultimate load to failure and stiffness of native anterior cruciate ligament.*

| Reference *Age Group* | Ultimate Load | Stiffness (N/mm) |
|---|---|---|
| Noyes and Grood[9] | | |
| 16 - 26 years | 1730 ± 660 | 182 ± 56 |
| 48 - 86 years | 734 ± 266 | 129 ± 39 |
| Woo et al[10] | | |
| 22 - 35 years | 2160 ± 157 | 242 ± 28 |
| 40 - 50 years | 1503 ± 83 | 220 ± 24 |
| 60 - 97 years | 658 ± 129 | 180 ± 25 |
| Rowden et al[11] | | |
| under 42 years | 2195 ± 427 | 306 ± 80 |

In a similar test, Race and Amis[12] measured the tensile strength of the anterolateral band of the PCL in ten donors ranging from 53 to 98 years of age resulting in a mean load to failure of 1600 N. The authors postulate that the combined anterolateral/posteromedial PCL bundle exhibits an ultimate load to failure of 1867 N.

Notably, an inverse relationship between ACL strength and age was observed.[10] The strength of the ACL of young specimens was approximately 40% higher than that of middle-aged and 225% higher than that of older specimens. Likewise, younger specimens showed a statistically significant higher linear stiffness than older specimens by 34%. However, it should be noted that all anatomically oriented data points up to age 75 in this study met or exceeded the 454 N threshold. Also, again please note that the grafts used in these studies do not necessarily represent grafts that would be used in the clinical setting. Tissue banks screen allografts for microbial contamination and concerns upon visual and physical inspections. Thus, tissue banks would use a more clinically suitable subset of the grafts than represented in Table 2.

## Impact of Donor Age on the Properties of Allograft Soft Tissue

Allograft tissues have been used for clinical sports medicine applications since the mid-1980s. Allografts have found their greatest acceptance in revision surgeries when autogenic grafts are difficult to obtain or no longer available. Nonetheless, since allogenic tissue eliminates surgical morbidity associated with autograft harvest, allografts are increasingly being used for primary reconstructions as well.[2,13] Allografts for sports medicine applications are recovered from a variety of sites and include the patellar tendon, the semitendinosus tendon, the tibialis tendon (both anterior and posterior), the gracilis tendon, and the Achilles tendon.

This variety provides a larger choice of tissue types and sizes, which is especially relevant in revisions in which bone may be deficient. It also allows the selection of graft types with the ability to tolerate high levels of stress. Various investigators have reported the patellar tendon to be 138 to 170% stronger and 125% stiffer than the native ACL.[4,14,15] In addition, the strength of the quadrupled semitendinosus/gracilis (hamstring) graft construct has been shown to be approximately 200% stronger and 300% stiffer than the native ACL.[4,11,14] To address the impact of donor age on tissue properties, Blevins et al[16] evaluated the effect of donor age on the mechanical properties of patellar tendons. The authors found no significant correlation between tensile strength and donor age, and hypothesized that "as a result of greater and more consistent stress being placed on the patellar tendon with increasing age, deterioration in its material properties is delayed and is less extensive than that seen in the ACL."

This finding has been corroborated by Bianchi, et al.[17] who found that patellar ligaments from donors 36 to 50 years and 51 to 65 years in age demonstrated insignificant difference both in load to failure and stiffness. The authors concluded that the tensile strength of patellar tendons across all age ranges exceeds the clinically recommended strength required for ACL reconstruction.

In further support, Flahiff, et al.[18] studied patellar tendon allografts for donors aged from 15 to 55 years. The results of their biomechanical analysis led them to conclude that "'there was no significant correlation between age and any of the mechanical properties.".

Lewis and Shaw[19] found that donor age had an insignificant effect on the stiffness of the Achilles tendon. The stiffness of tendons from donors between the ages of 36 and 50 years was measured at 794 ± 298 N/mm, whereas tendons from 79 to 100 year old donors was shown to be 634 ± 300 N/mm. Lewis and Shaw also calculated tendon stress, which is the force per unit area of the tendon cross-section, for various donor age groups; these data are presented in Table 3. Whereas the results are very similar for the 36 to 50 and 52 to 67 age groups, a donor age of 79 years and older had a moderately negative effect on tendon stress.

**Table 3:** *Tendon stress of the Achilles Tendon[19]*

| Donor Age Group | Stress ($N/mm^2$) | Stiffness (N/mm) |
|---|---|---|
| 36 - 50 | 73 ± 8 | 794 ± 298 |
| 52 - 67 | 73 ± 13 | 741 ± 70 |
| 79 - 100 | 48 ± 16* | 634 ± 300 |

**Significantly different, student's t-test $p<0.05$*

Gatt et al[20] investigated the effect of donor age on a variety of clinical quality soft tissue allografts that are used for ACL reconstruction from donors ranging in age from 15 to 87 years. No statistically significant differences for different age groups in either ultimate load to failure or in tendon stiffness could be demonstrated. In addition, the authors point out that the length of each of the grafts tested in this study was adequate for doubling into a two-stranded construct. Such a construct would thus far exceed the native ACL both in strength and stiffness.

More recently, a study was reported by Dr. Charles Brown of Brigham and Women's Hospital, Boston[21] which investigated the effects of donor age on the tensile properties of single strand and double strand tibialis tendon allografts that were subjected to Allowash XG®. For this study, Allowash XG®

tendons were processed utilizing tissue disinfection and cleaning technologies developed, validated and patented by LifeNet Health.[22,23] Tibialis tendon allografts were divided into three groups based on donor age; the age groups were 15 to 45 years (20 tendons), 46 to 55 years (26 tendons) and 56 to 65 years (20 tendons). The tendons in each group were further randomly assigned to tensile testing in either a single-strand or double-strand configuration. Cross-sectional tendon area for tendon stress calculation was measured using an area micrometer. For mechanical testing, each tendon was mounted in fixtures using tendon freezing clamps and was pre-conditioned for 100 cycles with a 50 to 250 N cyclic load. Each tendon was then extended to failure at a strain rate of 100% elongation per second. Tendon load to failure, stiffness, and displacement at failure (deformation) were determined based on the measured load-elongation curve. The data was analyzed within each age group using a non-parametric Mann-Whitney test. A significant difference was defined as $p<0.05$.

**No statistically significant difference was observed for ultimate load to failure, stiffness, stress, or displacement at failure for single strand tibialis tendons among the three age groups.** Both load to failure and stiffness in all three age groups indicate that single strand tibialis tendons are stronger than the native ACL based on published data (Table 1). An overview of the results for tibialis tendons in the single strand configuration is presented in Table 4. Also note that there is no significant effect of donor age on tissue properties.

Furthermore, evaluation of the tibialis tendons in the double-strand configuration also showed no statistically significant difference among the three age groups in regard to ultimate load to failure, stiffness, and displacement at failure (Table 5).

**Table 4:** *Average ultimate load to failure, stiffness, stress, and displacement at failure of tibialis tendons (single strand) processed with Allowash XG as a function of age.[21] For comparison, literature values for native ACL are added as derived from Table 2.*

| Donor Age Group (years) | Ultimate Load to Failure (N) | Stiffness (N/mm) | Stress (N/mm²) | Displacement at Failure (mm) |
|---|---|---|---|---|
| 15 to 45 | 3062 ± 699 | 569 ± 107 | 127 ± 28 | 6.1 ± 1.3 |
| 46 to 55 | 2729 ± 995 | 630 ± 213 | 104 ± 21 | 5.2 ± 1.7 |
| 56 to 65 | 3004 ± 603 | 525 ± 58 | 111 ± 16 | 6.8 ± 1.0 |
| Native ACL (see Table 2) | Range 658-2195 | Range 129-306 | na | na |

**Table 5:** *Average ultimate load to failure, stiffness, stress, and displacement at failure of tibialis tendons (double strand) processed with Allowash XG.[21] For comparison, literature values for native ACL are added as derived from Table*

| Donor Age Group (years) | Ultimate Load to Failure (N) | Stiffness (N/mm²) | Stress (N/mm²) | Displacement at Failure (mm) |
|---|---|---|---|---|
| 15 to 45 | 5124 ± 1206 | 886 ± 194 | 113 ± 18 | 6.9 ± 1.2 |
| 46 to 55 | 5262 ± 845 | 943 ± 190 | 103 ± 12 | 7.0 ± 1.5 |
| 56 to 65 | 5334 ± 1353 | 966 ± 202 | 103 ± 18 | 7.0 ± 1.6 |
| Native ACL (see Table 2) | Range 658-2195 | Range 129-306 | na | na |

## Impact of Sterilization Using Gamma Radiation on the Properties of Soft Tissue

As reviewed by Jon Block, the impact of irradiation on tissue grafts has been explored with a range of results.[24] However, different methodologies and methods of irradiation make comparisons difficult. Recent improvements in sterilization technologies[25], the continued stringent oversight of regulatory bodies, and the strict adherence of reputable tissue banks to regulations, all contribute to the continued advancement of safety of allograft tissue and development of controlled and well understood sterilization methods.[24] In 2004, LifeNet Health introduced the proprietary, comprehensive, and validated Allowash XG sterilization process, which encompasses control of incoming bioburden, removal of microorganisms through a controlled and patented cleaning and disinfection system,[22] and terminal sterilization of allograft tissue at low temperature. The process of terminal sterilization ensures that all allograft tissue supplied by LifeNet Health are treated to remove and inactivate bacteria and other viable and detectable infectious agents.[24] In a recently reported study,[26] the biomechanical properties of soft tissue grafts processed with Allowash XG sterilization process were examined. The tissues tested were patellar ligaments, fascia lata, anterior tibialis tendons, and semitendinosis tendons. Each graft type was divided into two groups: one group was gamma irradiated at a low absorbed dose (18.3 to 21.8 kGy) and the other group a moderate absorbed dose (24.0 to 28.5 kGy). Non-irradiated grafts served as experimental controls. During testing, each graft was loaded to failure and the ultimate tensile strength was calculated. A *p* value of $p<0.05$ was considered statistically significant.

As seen in Figures 1 and 2, the tensile strengths of anterior tibialis tendons or semitendinosis tendons, respectively, which were irradiated at either low or moderate doses, were statistically comparable to the matched, non-irradiated controls. In addition, the modulus of elasticity was statistically comparable between the two groups indicating no significant impact of gamma irradiation.

**Figure 1:** *Effect of gamma irradiation on tensile strength of anterior tibialis tendon. No statistical differences between groups (control vs. low dose, p=0.812; control vs. moderate dose, p=0.055)*

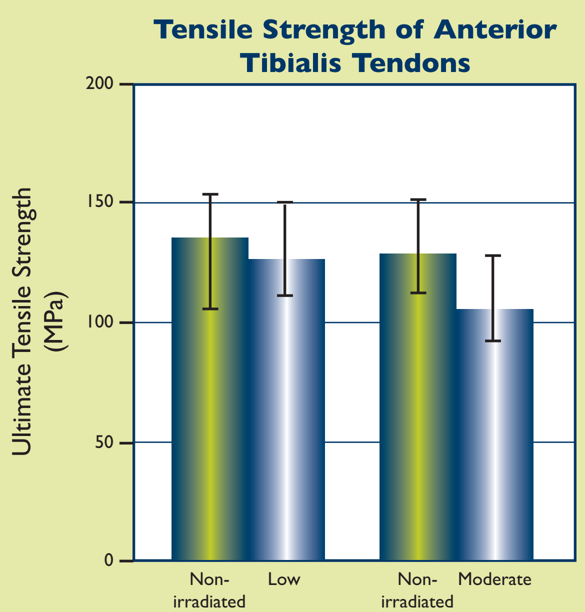
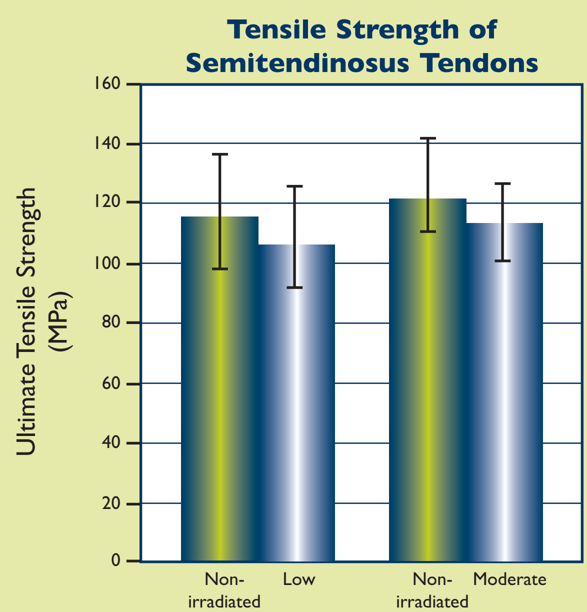
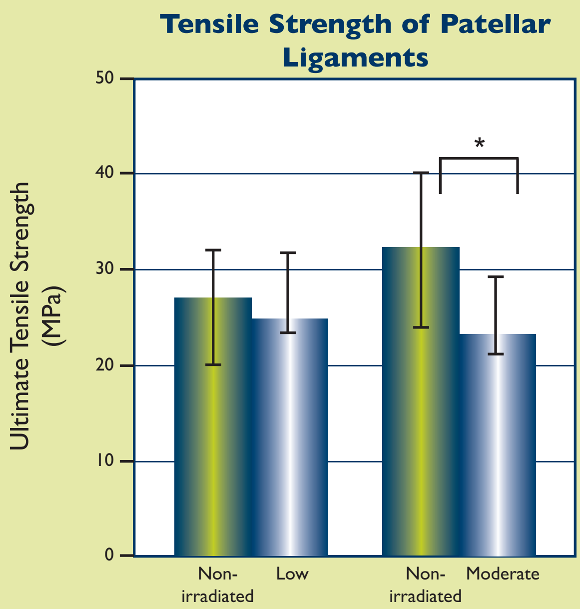



**Figure 2:** *Effect of gamma irradiation on tensile strength of semitendinosus tendon. No statistical differences between groups (control vs. low dose, p=0.543; control vs. moderate dose, p=0.160)*



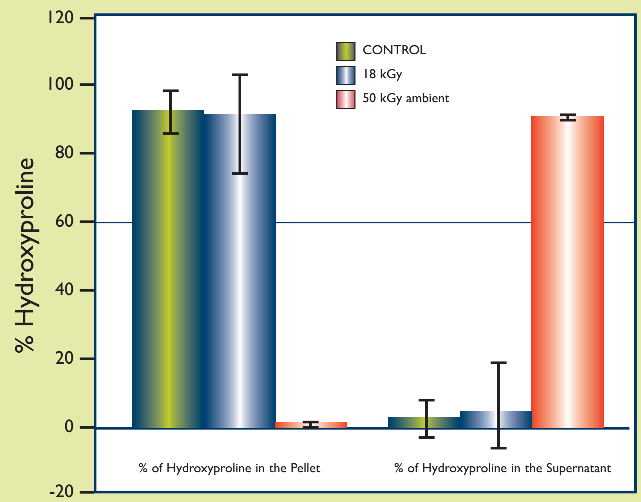
As seen in Figure 3, the tensile strengths of patellar ligaments irradiated at the low dose were likewise statistically comparable to the matched controls, however, patellar ligaments irradiated at a moderate dose showed a slightly reduced tensile strength than the matched non-irradiated grafts, perhaps because those controls were much higher than those seen for the low dose sample controls.

**Figure 3:** *Effect of gamma irradiation on tensile strength of patellar ligament bone-ligament-bone graft. No statistical difference was demonstrated, however, between control and moderate dose groups (p=0.016), although the non-irradiated matched group appeared unusually strong.*




In another study, presented at the 2003 International Atomic Energy Commission Meeting on Sterilization,[27] similar results were demonstrated using tibialis tendons. Furthermore the study evaluated the molecular structure, testing susceptibility to enzymatic digestion, which is a sensitive assay to measure irradiation-induced damage at the molecular level. The results (see Figure 4) demonstrated no statistical significant difference in the enzyme susceptibility of Allowash XG-processed tendons compared to the control group.

**Figure 4:** *Chymotrypsin sensitivity of Allowash® non-irradiated processed tendons compared to Allowash XG®-processed and gamma irradiated tendons.*



As mentioned earlier, a group at Brigham and Women's Hospital, Boston, headed by Dr. Charles Brown investigated the effect that donor age might have on the biomechanical properties of both Allowash®-processed (non-irradiated) and Allowash XG-processed (gamma irradiated) sterilized soft tissue.[21] Tensile testing was performed on tendons that were randomly assigned to a single-strand or double-strand configuration and both Allowash-processed (non-irradiated) and Allowash XG-processed (gamma-irradiated) tissues were evaluated.

As shown in Table 6, no statistically significant differences were observed for ultimate load to failure, stiffness, stress, or displacement at failure for single strand tibialis tendons between Allowash-processed and Allowash XG-processed tissue. An overview of the results for the non-irradiated tibialis tendons in the single strand configuration is presented in Table 6.

**Table 6:** *Average ultimate load to failure, stiffness, stress and displacement at failure of tibialis tendons as a function of age (single strand).[21] For comparison, literature values for native ACL are added as derived from Table 2.*

| Donor Age Group | Ultimate Load to Failure (N) | | Stiffness (N/mm) | | Stress (N/mm²) | | Displacement at Failure (mm) | |
|---|---|---|---|---|---|---|---|---|
| | Allowash® | Allowash XG® | Allowash® | Allowash XG® | Allowash® | Allowash XG® | Allowash® | Allowash XG® |
| 15 - 45 | 2843 ± 694 | 3062 ± 699 | 587 ± 105 | 569 ± 107 | 105 ± 18 | 127 ± 28 | 6.1 ± 1.6 | 6.1 ± 1.3 |
| 46 - 55 | 2823 ± 573 | 2729 ± 995 | 639 ± 174 | 630 ± 213 | 102 ± 21 | 104 ± 21 | 5.6 ± 1.4 | 5.2 ± 1.7 |
| 56 - 65 | 2988 ± 787 | 3004 ± 603 | 698 ± 138 | 525 ± 58 | 100 ± 24 | 111 ± 16 | 5.9 ± 1.7 | 6.8 ± 1.0 |
| Native ACL (see Table 2) | Range 658-2195 | | Range 129-306 | | na | | na | |

As shown in Table 7, evaluation of the tibialis tendons in the double-strand configuration showed no statistically significant differences with regard to ultimate load to failure, stiffness, and displacement at failure between Allowash®-processed (non-irradiated) and Allowash XG®-processed (gamma-irradiated) tendons. The average stress at failure, however, was significantly lower for the irradiated tendon in the 56 to 65 age group than average stress measured in the other two age groups (p=0.035). This observation does not hold true for the gamma-irradiated tibialis tendons since results were not significantly different among the three age groups.

**Table 7:** *Average ultimate load to failure, stiffness, stress and displacement at failure of tibialis tendons as a function of age (double strand).[21] For comparison, literature values for native ACL are added as derived from Table 2.*

| Donor Age Group | Ultimate Load to Failure (N) | | Stiffness (N/mm) | | Stress (N/mm²) | | Displacement at Failure (mm) | |
|---|---|---|---|---|---|---|---|---|
| | Allowash® | Allowash XG® | Allowash® | Allowash XG® | Allowash® | Allowash XG® | Allowash® | Allowash XG® |
| 15 - 45 | 5074 ± 1032 | 5124 ± 1206 | 930 ± 185 | 886 ± 194 | 98 ± 18 | 113 ± 18 | 6.4 ± 0.7 | 6.9 ± 1.2 |
| 46 - 55 | 5255 ± 706 | 5262 ± 845 | 947 ± 139 | 943 ± 190 | 99 ± 18 | 103 ± 12 | 6.6 ± 0.7 | 7.0 ± 1.5 |
| 56 - 65 | 4971 ± 1980 | 5334 ± 1353 | 931 ± 223 | 966 ± 202 | 82 ± 12 | 103 ± 18 | 6.4 ± 1.5 | 7.0 ± 1.6 |
| Native ACL (see Table 2) | Range 658-2195 | | Range 129-306 | | na | | na | |

## Functional Outcomes

The in vitro data presented here supports the use of Allowash XG to clean, disinfect, and sterilize allograft tissue for sports medicine applications. In a recent retrospective clinical study, the research group of Drs. Harner and Dr. Fu at the UPMC Center for Sports Medicine in Pittsburgh compared the clinical outcome of anterior cruciate ligament (ACL) reconstruction with irradiated allograft to autograft bone–patellar tendon–bone (BPTB).[3] Allograft BPTBs were sourced from a single tissue bank and treated with 25 kGy gamma radiation. Patients were evaluated at an average follow-up of 4.2 years (range: 1.8 to 8.4 years). The investigators found that patients undergoing ACL reconstruction with irradiated allograft BPTB had similar clinical outcomes to those reconstructed with autograft BPTB. ***The data suggest that gamma irradiation up to 25 kGy can safely be used to sterilize allograft tendons without adversely affecting clinical outcome.***

## Conclusion

Results of recent studies evaluating the effect of donor age on the mechanical properties of a variety of soft tissue grafts suggest that the use of tissue from donors up to at least the age of 70 for ACL and PCL reconstruction is justified.

**In addition, results of recent studies also indicates that when using a validated methodology, controlled low-dose temperature gamma irradiation can be used to sterilize allografts. Taken as whole, the peer-reviewed literature, pre-clinical testing and clinical outcome data all indicate that when performed under controlled conditions, the Allowash XG process does not adversely affect the biomechanical or biochemical properties of soft tissues needed for their intended clinical applications.**

When making their choice among tissue suppliers, clinicians seek to find a balance between utmost tissue safety and greatest tissue efficacy in order to achieve the best patient outcome possible. With allograft tissue processed with Allowash XG technology, LifeNet Health is able to satisfy both needs. Today, it is more critical than ever for physicians and hospital administrators to rely on sterile tissue provided by well-known, accredited tissue banks such as LifeNet Health. With Allowash XG, LifeNet Health takes tissue safety to the next level.

Since 1995, LifeNet Health has delivered over 2 millions allografts to the medical community with no incidence of disease transmission.

## References


1. Mologne TS and Friedman MJ: Graft options for ACL reconstruction. Amer J Orthop 29: 845-853, 2000.

2. McGuire DA and Wolchok JC: Allografts for ligamentous reconstruction of the knee. Tech Knee Surg 2: 166-183, 2003.

3. Rihn JA, Chhabra A, Fu FH et al: Does irradiation affect the clinical outcome of patellar tendon allograft ACL reconstruction? Presented at the American Orthopaedic Society for Sports Medicine Annual Meeting; Keystone, CO; July 2005.

4. Noyes FR, Butler DL, Grood ES, et al: Biomechanical analysis of human ligament grafts used in knee-ligament repairs and reconstructions. J Bone Joint Surg 66A: 344-352, 1984.

5. Morrison JB: Bioengineering analysis of force actions transmitted by the knee joint. Biomed Eng (April): 164, 1968.

6. Morrison JB: Function of the knee joint in various activities. Biomed Eng 4: 573-580, 1969.

7. Morrison JB: The mechanics of the knee joint in relation to normal walking. J Biomech 3: 51-61, 1970.

8. Gulick DT and Yoder HN: Anterior cruciate ligament reconstruction: clinical outcomes of patella tendon and hamstring tendon grafts. J Sports Sci Med 1: 63-71, 2002.

9. Noyes FR and Grood ES: The strength of the anterior cruciate ligament in humans and Rhesus monkeys. J Bone Joint Surg 58:1074-1082, 1976.

10. Woo SL, Hollis JM, Adams DJ et al: Tensile properties of the human femur-anterior cruciate ligament-tibia complex. Amer J Sports Med 19: 217-225, 1991.

11. Rowden N, Sher A, Rogers GJ et al: Anterior cruciate ligament graft fixation. Initial comparison of patellar tendon and semitendinosus autografts in young fresh cadavers. Amer J Sports Med 25: 472-478, 1997.

12. Race A and Amis AA: The mechanical properties of the two bundles of the human posterior cruciate ligament. J Biomech 27: 13-24, 1994.

13. Tom JA and Rodeo SA: Soft tissue allografts for knee reconstruction in sports medicine. Clin Orthop 402: 135-156, 2002.

14. Cooper DE, Deng XH, Burstein AL et al: The strength of the central third patellar tendon graft. Amer J Sports Med 21: 818-824, 1993.

15. Brown CH and Sklar JH: Graft selection: nonpatellar alternatives gain popularity. Biomechanics (June): 21-25, 1998.

16. Blevins FT, Hecker AT, Bigler GT et al: The effects of donor age and strain rate on the biomechanical properties of bone-patellar tendon-bone allografts. Amer J Sports Med 22: 328-333, 1994.

17. Bianchi JR, Summitt M, Scurti G et al: The influence of donor factors on the mechanical properties of pre-shaped bone-patellar tendon-bone allografts. Presented at the Annual Meeting of the American Society of Biomechanics, Abstract No. 067; San Diego, CA; August 2001.

18. Flahiff CM, Brooks AT, Hollis JM et al: Biomechanical analysis of patellar tendon allografts as a function of donor age. Amer J Sports Med 23: 354-358, 1995.

19. Lewis G and Shaw KM: Tensile properties of human tendon AchillisAchilles: Effect of donor age and strain rate. J Foot Ankle Surg 36: 435-445, 1997.

20. Gatt CJ, Savage J, Lauer B et al: The effect of donor age on the mechanical properties of soft tissue allografts used for ACL reconstruction. Presented at 51st Annual Meeting of the Orthopaedic Research Society, Paper No: 0061; 2005.

21. Greaves LL, Hecker AT, Brown CH. The effects of donor age and low dose gamma irradiation on the initial biomechanical properties of human tibialis tendon allografts. The American Journal of Sports Medicine, 36:1358-1366 (2008).

22. United States Patents- 5,556,379; 5,820,581; 5,977,034; 6,024,735. LifeNet Health introduced its patented Allowash® Technology, a process for cleaning and disinfection of allograft tissue, in 1995. Allowash® Technology is specially designed to facilitate the removal of cellular elements from musculoskeletal tissue to reduce the potential danger of disease transmission while maintaining structural integrity. During the Allowash® process a combination of detergents in the "cleaning" steps removes bone marrow and other cellular components associated with bone. Hydrogen peroxide and alcohol processing steps further reduce potential bioburden by acting as disinfectants. As advancements in technology are achieved, LifeNet Health's Allowash® Technology is continually reengineered to further increase the safety of the company's allograft tissue. A result of these developments is Allowash XG®™, a process that offers sterility without compromising the biochemical or biomechanical properties of allografts used for surgical applications. Additional information on Allowash® Technology and Allowash XG®™ can be found on LifeNet Health's Web site at www.accesslifenet.org.

23. Moore M, Linthurst Jones A, Gaskins B et al.: Adaptation of ANSI/AAMI/ISO 11137 method 2B sterilization validation for medical devices to tissue banking. Presented at American Association of Tissue Banks Annual Meeting; Chicago, IL; August 2004.

24. Block JE: The impact of irradiation on the microbiological safety, biomechanical properties and clinical performance of musculoskeletal allografts. Orthopedics. 2006 Nov;29 (11):991-6.

25. Tomford WW: Bone allografts: past, present and future. Cell Tissue Bank 1: 105-109, 2000.

26. Balsly CR, Cotter AT, Williams LA, Gaskins BD, Moore MA, Wolfinbarger LW. Effect of low dose and moderate dose gamma irradiation on the mechanical properties of bone and soft tissue allografts. J Cell Tissue Banking, April 23, 2008.

27. Wolfinbarger L Jr: Gamma irradiation of allograft tendons. Presented at International Atomic Energy Commission Meeting on Sterilization of Tissues Using Radiation; Wrexham, Wales UK; September 2003.

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