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Allograft Tissue Sterilization Using Allowash XG[®]

Allograft Tissue Sterilization Using Irradiation— What are the Implications for Clinical Performance?

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Musculoskeletal allograft tissue is typically procured and processed aseptically to avoid contamination with microorganisms. Nonetheless, aseptic handling of tissue does not eliminate the risk of tissue contamination or the potential for disease transmission nor does it render the tissue sterile. For that reason, many tissue banks have introduced additional measures to maximize the safety of transplanted allografts.^{1,2,3}

One such measure is the development of sterilization systems that include a combination of sterilants to ensure the best possible results. Gamma irradiation is currently the most popular option for the terminal sterilization of allograft tissue and is commonly applied after the final processing step.⁴ Complete tissue sterilization can be achieved with gamma radiation when used in sufficiently high doses.¹ However, an excessively high dose of uncontrolled radiation has been shown to have a deleterious effect on the material properties of most allograft tissue, particularly of structural allografts, and is therefore generally not recommended for processing of allogenic tissue.^{5,6,7}

The temperature at which radiation is administered appears to play a critical role as well. Studies have shown that uncontrolled gamma irradiation of freeze-dried or hydrated samples at room temperature negatively affects biomechanical tissue properties.⁹ In contrast, irradiated deep-frozen bone allografts seem to be less brittle than similar grafts irradiated at room temperature.^{8,9} Tissue banks have incorporated this finding into the development of improved sterilization methods for allograft tissue and now carry out sterilization at low temperatures.

Several pre-clinical and clinical studies have been undertaken that demonstrate the safety of controlled-dose, low-temperature gamma irradiation with both soft and hard tissues. This article describes selected studies that have investigated the effect of this sterilization methodology on various allograft types.

IMPACT OF STERILIZATION USING ALLOWASH XG® ON THE PROPERTIES OF SOFT TISSUE

In a research study performed at LifeNet Health, the biomechanical properties of soft tissue grafts processed with LifeNet Health's Allowash XG®, which includes a terminal sterilization step on dry ice, were examined. The tissues tested were patellar ligaments, fascia lata, anterior tibialis tendons, and semitendinosus tendons. Each graft type was divided into two groups: one group was gamma irradiated at a targeted absorbed dose of 18 kGy (18.3 to 21.8 kGy) and the other group received a targeted absorbed dose of 25 kGy (24 to 28.5 kGy). Allowash®-processed (non-irradiated) grafts served as experimental controls.

During testing, each graft was loaded to failure and the ultimate tensile strength was calculated. The tensile strength of fascia lata, anterior tibialis tendons, and semitendinosus tendons irradiated at both the target absorbed doses of 18 and 25 kGy was statistically comparable to the matched grafts in the control group (Figures 1 and 2). A p value of $p < 0.05$ was considered statistically significant.

Tensile Strength of Donor Matched Anterior Tibialis Tendons

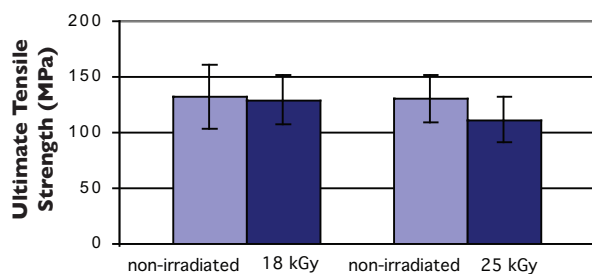


Figure 1. Tensile strength of gamma irradiated (18 and 25 kGy) tibialis tendons compared to non-irradiated tendons (18 kGy: $p=0.812$; 25 kGy: $p=0.055$).¹⁰

Tensile Strength of Donor Matched Semitendinosus Tendons

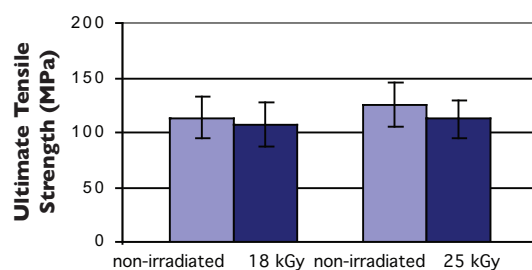


Figure 2. Tensile strength of gamma irradiated (18 and 25 kGy) semitendinosus tendons compared to non-irradiated tendons (18 kGy: $p=0.543$; 25 kGy: $p=0.16$).¹⁰

The tensile strength of patellar ligaments irradiated at the target absorbed dose of 18 kGy was likewise statistically comparable to the matched controls, however, those patellar ligaments irradiated at 25 kGy showed a reduced tensile strength compared to the matched non-irradiated grafts. Interestingly, the non-irradiated control grafts that were matched to the latter group demonstrated a significantly higher tensile strength, which may have contributed to the significant difference between the patellar ligaments irradiated at 25 kGy and the corresponding control grafts (Figure 3).¹⁰

The results of laboratory experiments do not always provide a good indication of actual clinical outcome, however. In a recent prospective clinical study, the research group of Drs. Harner and 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).¹¹ Allograft BPTBs were sourced from a single tissue bank (LifeNet Health), processed using Allowash® technology, and sterilized at 25 kGy using 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.**

Interestingly, the patients who underwent allograft reconstruction were older (44 ± 8.4 versus 25.3 ± 9.3 years) and had a longer median time from injury to surgery (17.1 versus 9.7 weeks) than autograft recipients. The authors report that there were no differences in knee-specific or general measures of health status between patients who received allograft and autograft BPTBs. Statistically adjusting for differences in age and time of surgery did not change this conclusion.¹¹

THE EFFECT OF ALLOWASH XG® ON HUMAN TENDON ALLOGRAFTS

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 non-irradiated and gamma radiation sterilized soft tissue.¹² Tibialis tendon specimens were divided into three groups based on the age of the tissue donor. The age groups selected were 15 to 45 years, 46 to 55 years, and 56 to 65 years of age. Tensile testing was performed on tendons that were randomly assigned to a single-strand or double-strand configuration, and both non-irradiated (Allowash®-processed) and gamma-irradiated (Allowash XG®-processed) tissue were evaluated.

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 (non-irradiated) and Allowash XG®-processed (gamma-irradiated) tissue. This observation holds true for all three age groups. An overview of the results for tibialis tendons in the single strand configuration is presented in Table 1. (next page)

Evaluation of the tibialis tendons in the double-strand configuration showed no statistically significant differences in regard to ultimate load to failure, stiffness, and displacement at failure between Allowash®-processed (non-irradiated) and Allowash XG®-processed (gamma-irradiated) tendons. The result for average stress at failure, however, was significantly lower for the non-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 2). The difference between non-irradiated and gamma-irradiated tendons in the 56 to 65 age group in this study may be a consequence of the generation of free radicals,

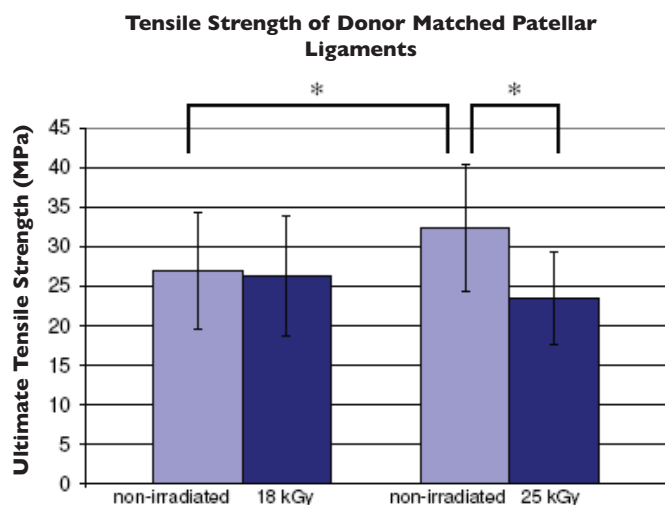


Figure 3. Tensile strength of gamma irradiated patellar ligaments (18 and 25 kGy) compared to non-irradiated ligaments (18 kGy: $p=0.86$; 25 kGy: $p=0.016$).¹⁰ Asterisks denote a statistically significant difference as described in text

which can cause minor cross-linking of collagen fibers¹³, which in turn could lead to a change in the tensile strength. To reduce the potential for elongation of irradiated grafts Curran and colleagues recommend pre-tensioning grafts before implantation.¹⁴

BIOMECHANICAL STRENGTH OF BONE ALLOGRAFTS FOR SPINE SURGERY FOLLOWING ALLOWASH XG® STERILIZATION

The effect of gamma radiation on the mechanical strength of structural grafts used for such applications as spinal fusion surgery has been the topic of several studies.^{9,15} A study was performed with three VertiGraft® allograft types, VG2® Cervical (VG2C), VG2® PLIF, and VG1® ALIF, to characterize the biomechanical properties of cortical and cortico-cancellous bone tissue processed with Allowash XG® at a target absorbed irradiation dose of 15 kGy.¹⁶ The grafts were tested in axial compression, compressive shear, and static torsion following ASTM Standard F2077 guidelines (FDA ASTM F2077-03, 2006).¹⁷ Non-irradiated Allowash® treated grafts were used as controls.

With all three VertiGraft® allograft types, no statistically significant differences were found between the irradiated and the control grafts when tested in axial compression and compressive shear (Figures 4 and 5). Although the average torsional strength of the Allowash XG treated VG2 Cervical

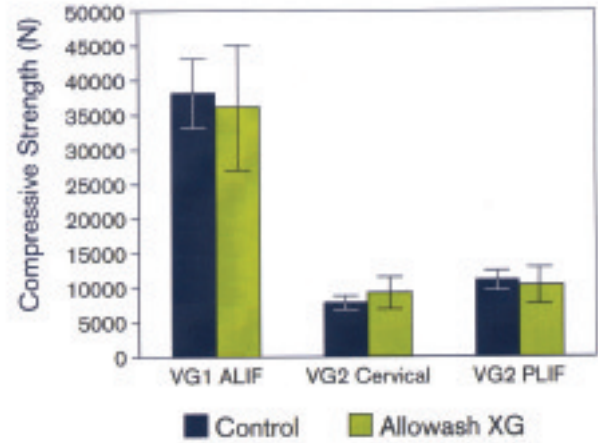


Figure 4. Compressive strength of Allowash XG® processed (15 kGy irradiation dose) VertiGraft compared to non-irradiated control.¹⁶

allograft was statistically significantly lower than the corresponding control, the irradiated tissue exceeded the base level value for interbody device strength.¹⁸

The results of this study were confirmed in a parallel study conducted by LifeNet Health using the four VertiGraft allograft types, VG2 Cervical (VG2C), VG2 PLIF, VG1 ALIF, and VG1 Fibular (VG1 FIB). Grafts were Allowash XG processed and irradiated at target absorbed doses of 18 kGy and 25 kGy; control grafts were Allowash-processed and not irradiated. Grafts were tested for static compression and compressive shear and compared to the compressive load and torque that the cervical spine and lumbar spine are subjected to in vivo.

Table 1. Average ultimate load to failure, stiffness, stress, and displacement at failure of tibialis tendons (single strand).¹²

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

Table 2. Average ultimate load to failure, stiffness, stress, and displacement at failure of tibialis tendons (double strand).¹²

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

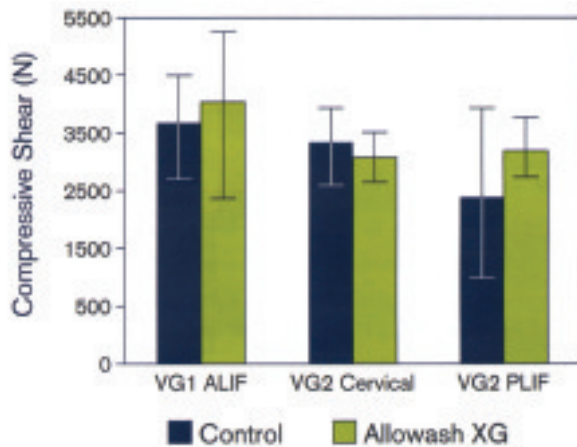


Figure 5. Compressive shear of Allowash XG® processed (15 kGy irradiation dose) VertiGraft compared to non-irradiated control.¹⁶

Neither 18 kGy nor 25 kGy absorbed irradiation doses negatively affect the required biomechanical strength of VertiGraft for application in the clinical setting.¹⁰

In addition to “machined grafts” such as the VertiGraft constructs, traditional structural grafts are often used for spinal fusion surgery as well. The effects of Allowash XG processing on the biomechanical properties of the traditional weight-bearing allograft types iliac crest wedge and Cloward dowel grafts were also examined in a laboratory experiment. Again, Allowash XG processing with target absorbed radiation doses of 18 and 25 kGy was administered to the study grafts on dry ice; matched non-irradiated, Allowash-processed grafts were used as controls.

During testing, each graft was loaded to failure and the ultimate compressive strength was calculated. The compressive strength of iliac crest wedges and of Cloward dowels irradiated at both the target absorbed dose of 18 and 25 kGy was statistically comparable to the matched

grafts in the control group (Figures 6 and 7). This study revealed that the use of terminal sterilization at an absorbed dose between 18 and 25 kGy as part of the Allowash XG process does not yield a significant change in biomechanical strength compared to the non-irradiated control.¹⁰

ALLOWASH XG® PROCESSED BONE ALLOGRAFTS FOR STRUCTURAL DEFECTS – NO LOSS OF COMPRESSIVE STRENGTH

Durable bone grafts such as shafts and cortical rings and struts are typically used for the reconstruction of large osseous defects that result from such procedures as fracture management, general orthopaedic surgery, total joint arthroplasty procedures, and spine surgery. The biomechanical properties of these grafts are of particular concern since durable bone grafts are generally utilized in load bearing applications that require high structural integrity. Due to the relatively large size of many durable bone grafts (greater than 12 cm in length), processing techniques used by tissue banks must also be adjusted to accommodate these grafts.

A major complication of implanted large durable bone allografts is the risk of fracture. The review of outcomes of patients who received massive structural allografts has shown that the overall incidence of graft fracture under normal loading conditions typically lies well below 20%.^{7,19,20} In a clinical study performed by Hernigou and colleagues²¹, the results of 127 massive allografts sterilized by gamma irradiation with 25 kGy and followed for a minimum of three years were reported. Fracture complications were found in 6% of the patients followed, suggesting that irradiation does not significantly affect clinical outcome.²¹

Numerous groups have conducted studies to investigate the embrittlement of cortical bone tissue and consequent

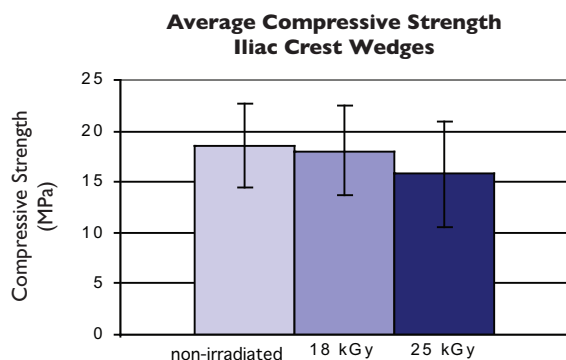


Figure 6. Compressive strength of gamma irradiated (18 and 25 kGy) iliac crest wedges compared to non-irradiated control grafts (18 kGy: $p=0.754$; 25 kGy: $p=0.095$).¹⁰

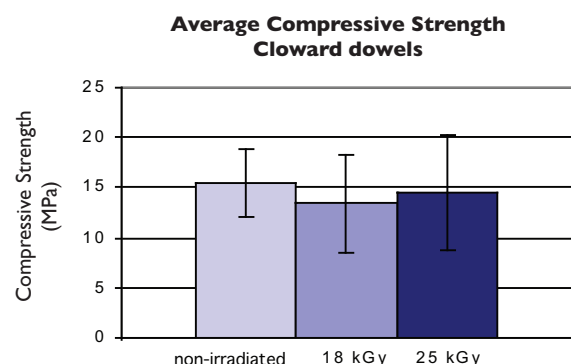
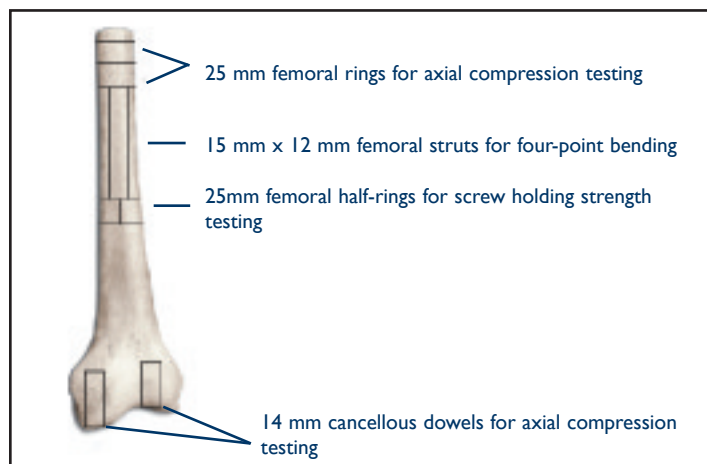


Figure 7. Compressive strength of gamma irradiated (18 and 25 kGy) Cloward dowels compared to non-irradiated control grafts (18 kGy: $p=0.159$; 25 kGy: $p=0.551$).¹⁰

fatigue fracture formation and reduction of biomechanical functionality in a laboratory setting.^{7,8,22,23} It is a generally accepted premise that gamma irradiation causes the destruction of collagen alpha chains, most likely mediated by reactive free hydroxyl radicals generated by radiolysis of water, thus resulting in increased bone brittleness.²⁴ Hamer et al.⁸ observed that cortical bone rings irradiated at 30.2 kGy on dry ice was significantly less brittle than corresponding samples irradiated at room temperature. Load at failure for specimens irradiated at room temperature was 84% of the value of the tissue irradiated on dry ice. Nonetheless, the groups of Mitchell et al.⁷ and Akkus and Belaney²³ showed in bench studies that cortical bone irradiated at averages doses of between 31.7 kGy and 36.4 kGy may be more predisposed to fatigue fracture formation than non-irradiated cortical tissue. These groups, therefore, recommend that structural bone grafts be designed to minimize functional strains and avoid stress risers to prevent premature fatigue failures.^{7,23}

A study performed at LifeNet Health investigated the effect of a sterilizing dose of gamma radiation on the biomechanical properties of durable bone grafts processed using Allowash® technology. Seven distal femur pairs and three fibular shaft pairs were used for this study. One graft from each matched pair served as the experimental group and was irradiated on dry ice at an absorbed dose of 40.4 kGy to 46.4 kGy. The corresponding non-irradiated, Allowash®-processed grafts were used as controls. The absorbed dose of 40.4 kGy to 46.4 kGy was selected to simulate a worst-case scenario; a gamma radiation dose of 26.4 kGy had been previously calculated as the minimum sterilizing dose based on ANSI/AAMI/ISO Method I Protocol sterilization validation studies modified for human tissue.¹⁰

Fibular shafts were cut into 20 mm segments for axial compression testing. Distal femurs were cut into the



following samples for biomechanical testing:

Axial compression was tested by loading the graft samples until failure, then dividing the measured force at failure by the cross-sectional area of the tested graft. The **results for all three graft types tested** (25 mm femoral rings, 14 mm cancellous dowels, and 20 mm fibular segments) **revealed no statistically significant difference between gamma-irradiation sterilized and non-irradiated tissue** ($p>0.079$).¹⁰

The fixation strength of screws used to secure allograft bone in a surgical site is often cited as the weakest point in allograft fixation^{25,26} and is directly related to the strength of the bone adjacent to the fixation screw. To test for screw fixation strength, cortical bone screws were inserted into femoral half-ring specimens and a screw-push-out test was performed. The maximum load required to extrude the screw normalized by tissue thickness was measured; **it was found that the difference between the gamma irradiated and non-irradiated samples was not statistically significant** ($p=0.779$).¹⁰

Finally, the bending strength of cortical struts cut from the femur was examined. Cortical struts are regularly used as a structural support in fracture and trauma surgery, and the most common mode of allograft failure in these positions is fracture of the graft. **The results of four-point bending testing between the non-irradiated control and the gamma-irradiated sample were compared and no statistically significant difference was found** ($p=0.144$).¹⁰

THE EFFECT OF STERILIZATION ON OSTEOINDUCTIVITY

The effect of a variety of sterilization methods on the osteoinductivity of demineralized bone matrix (DBM) has been extensively studied in various animal models implanted at both heterotopic and orthotopic sites.^{27,28,29,30,31} Of all sterilization methods examined, gamma irradiation demonstrated the most consistent results and appeared to be the most appropriate sterilizing method for demineralized bone in clinical applications.

In further studies, the effect of gamma radiation at various temperatures on bone formation and remodeling were explored. In experiments performed by Weintraub and Reddi²⁸, DBM samples that had been maintained in ice water and irradiated with doses up to 25 kGy showed inductive properties that were similar to the non-irradiated control. Dziedzic-Goclawska and colleagues²⁹ irradiated DBM at room temperature or on dry ice (-72°C). While samples that were irradiated at room



temperature had been completely resorbed five weeks after implantation into the muscle pouch of a rat, DBM preparations irradiated on dry ice were osteoinductive and were resorbed more slowly. DBM that had been treated with a dose of 35 kGy at -72°C demonstrated new bone formation that was comparable to non-irradiated control samples.²⁹

In light of the finding that DBM irradiated at low temperatures was less susceptible to radiation damage, LifeNet Health undertook a study to examine the results of a range of absorbed gamma radiation doses on demineralized bone particles.^{10,32} In this assay, DBM particles were irradiated at 7 kGy, 14 kGy, 21 kGy, and 30 kGy on dry ice. Experimental samples were implanted in muscle pouches of athymic mice for 28 days and were then compared to non-irradiated control samples in regard to percentage calcium deposition. The data demonstrated that gamma irradiation decreased the remineralization of the implanted DBM preparation to a point at which it allowed for greater resorption and replacement with new bone. This observation corroborates the results of experiments performed by Wientroub and Reddi, which suggested that irradiation of 30 to 50 kGy enhanced bone induction, leading to a higher level of mineralization than non-irradiated control samples in a heterotopic rat model.²⁸ The experimental samples irradiated at each dose did display significant osteo-inductivity, however, regardless of the dose administered.³²

CONCLUSION

Using a validated methodology, controlled dose, low-temperature gamma irradiation can be used to obtain

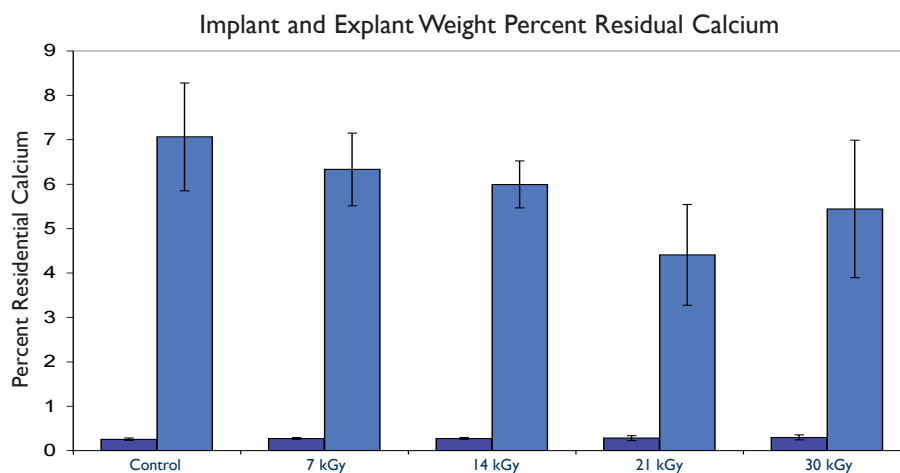
sterile allografts. Taken as a whole, peer-reviewed literature, pre-clinical testing, and clinical outcome data all indicate that when performed under controlled conditions regarding temperature as well as irradiation dose setting, **the Allowash XG® process does not adversely affect the biomechanical or biochemical properties of 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 that physicians and hospital administrators rely on sterile tissue provided by well-known, accredited tissue banks such as LifeNet Health. With Allowash XG®, LifeNet Health takes tissue safety and efficacy to the next level.

Since 1995, over 1.5 million allografts have been delivered to the medical industry and no incident of disease transmission has been directly linked to tissue screened and processed by LifeNet Health.

ABBREVIATIONS	
AAMI	Association for the Advancement of Medical Instrumentation
ACL	Anterior cruciate ligament
ANSI	American National Standards Institute
ASTM	American Society for Testing and Materials
BPTB	Bone–patellar tendon–bone
DBM	Demineralized bone matrix
FDA	Food and Drug Administration

Figure 7. Residual weight changes in percent residual calcium of DBM irradiated on dry ice.³²



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