The Impact of Irradiation on the Microbiological Safety, Biomechanical Properties and Clinical Performance of Musculoskeletal Allografts

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ABSTRACT

There is no generally accepted consensus in the published scientific literature regarding the safest and most effective radiation dose for treating musculoskeletal allografts prior to transplantation. A dose of 25 kGy of gamma irradiation has often been recommended as the "standard" dose for reducing the risk of tissue bacterial contamination. However, it has been demonstrated that this dose exceeds the dose necessary to provide complete bactericidal coverage (10–15 kGy). While this "standard" dose may have a moderate negative effect on the biomechanical properties of allografts, particularly grafts that are required to provide structural or functional support, allografts treated with lower doses do not suffer the same deleterious effects. Processing of donor samples should be performed aseptically including rigorous medical history screening and serological testing for viral contamination, as this is the most efficient means of identifying contaminated specimens. An irradiation dose less than 15 kGy should be used to eliminate residual risk of bacterial contamination in musculoskeletal allografts, which preserves the biomechanical properties of these tissues.

BACKGROUND AND RATIONALE

Hundreds of thousand of patients each year derive benefit from the use of musculoskeletal allografts for a variety of orthopedic and neurosurgical applications.[1-4] While allograft usage historically has been restricted to massive grafting procedures such as limb salvaging operations following osseous tumor resection, the frequency with which surgeons utilize musculoskeletal allografts has risen markedly over the past 15 years due, in large measure, to the substitution of processed cortical wedges, dowels, cancellous chips, and connective tissues such as patellar tendons for traditional autografts.[2] Indeed, surgeons now employ allografts routinely in complex reconstructive and revision surgeries where large bony voids are commonly encountered or where supplemental mechanical support is needed.[5] Additionally, demineralized allogeneic bone, due to its mildly osteoinductive property, has enjoyed increased use as a grafting matrix because it can effectively extend the limited supply of autograft.[6] The impetus to utilize musculoskeletal allografts has resulted from the growing awareness of the serious morbidity associated with harvesting autologous tissue coupled with renewed assurances provided by tissue banks that grafts are procured, handled and processed in a manner that attenuates, to an acceptable degree, the risk of contamination particularly when tissue banks adhere to guidelines set forth by the American Association of Tissue Banks and the US Food and Drug Administration.[4, 7]

Musculoskeletal allografts are generally harvested and processed aseptically to avoid adding contamination to tissues.[4] Standard processing steps include, but are not limited to, tissue washing to eliminate marrow, lipids and other antigenic cellular elements as well as the use of detergents, ethanol, surfactants, and antibiotic rinses.[2, 4] Freezing and freeze-drying (i.e., lyophilization) are commonly used for tissue storage.[1] Nonetheless, aseptic processing *per se* does not entirely remove all risk of tissue contamination.[1, 4] Consequently, many tissue banks also employ an additional measure, such as gamma irradiation, to provide a further assurance of microbiological safety.[1, 2, 4, 8, 9] In contrast to other methods of allogeneic tissue processing, irradiation has the singular distinction of providing complete tissue sterilization if the dose is sufficiently high.[9] Unfortunately, using high doses (e.g., > 40 kGy) of irradiation has a deleterious effect on the biomechanical and biologic properties of the tissue.[5, 10] Therefore, most tissue processors have had to accept a trade off between preserving allograft biomechanical integrity and providing a sufficient level of tissue sterility with lower doses of irradiation.

Unfortunately, there is lack of consensus regarding the lowest acceptable threshold dose of irradiation for treating musculoskeletal allografts prior to distribution and implantation. In a survey of 36 tissue banks, Vangsness et al[11] found that doses ranging from 10 to 35 kGy of

gamma irradiation are routinely used to sterilize allografts. An irradiation dose in the range of 15 to 25 kGy also has been promulgated as an industry standard based variously on studies published in the 1950s as well as on the typical dose used to sterilize medical instruments.[8,9] Regrettably, this currently recommended dose range has not been based on a clear, objective criterion or goal of irradiation in musculoskeletal tissue processing. This commentary seeks to clarify the impact of gamma irradiation treatment on musculoskeletal allografts and to offer recommendations with respect to an acceptable threshold dose based upon the need to preserve tissue biomechanical integrity and to reduce the risk of microbiological contamination.

THE EFFECTIVENESS OF IRRADIATION ON ALLOGRAFT MICROBIOLOGICAL SAFETY

The primary mechanism by which gamma irradiation provides bactericidal effects is via direct alteration of nucleic acids leading to genome dysfunction and destruction.[4] If performed at room temperature, irradiation of allogeneic tissue also results in the generation of free radicals, primarily from liquid water, which have a direct antimicrobial effect.[4,9] There is general agreement that a gamma irradiation dose of less than approximately 20 kGy provides complete elimination of bacterial contamination from musculoskeletal allografts.[5] In their classic study, Turner et al[12] demonstrated that a 10 kGy dose was effective in providing complete bactericidal coverage in frozen or freeze-dried cortical bone samples after contamination with a commonly encountered bacterium, *S. aureus*. Cohen[13] confirmed these findings in contaminated bone samples using a higher dose (20 kGy). Finally, DeVries et al[14] reported that only gram-positive cocci were resistant to a 10 kGy irradiation dose, but that all bacteria types were killed by 20 kGy. It should be stressed, however, that the microbiological safety of musculoskeletal allografts remains a function of the incoming bioburden of the material and whether each tissue bank employs stringent, validated tissue processing methods such as aseptic processing.

BIOMECHANICAL PROPERTIES OF IRRADIATED ALLOGRAFTS

Bone Allografts: Although treatment of musculoskeletal allografts with gamma irradiation markedly reduces the risk of contamination, tissues are invariably weakened by this process due to denaturation and, in some cases, destruction of collagen chains resulting in tissue embrittlement.[1,10] It is widely accepted that excessively high doses (30-90 kGy) of irradiation have a deleterious effect on the biomechanical properties (e.g., torsional, bending, compressive strength) of cortical allografts, particularly if the intended use of the graft is to provide structural support.[15-19] Consequently, structural allografts irradiated at these dose levels are contraindicated for implantation.

However, there is a greater lack of consensus with respect to the impact on biomechanical properties for doses ranging from 20 to 30 kGy or whether any measurable decrements in these properties have clinical relevance. For example, Bright et al[9] demonstrated that a 25 kGy dose did not affect the biomechanical properties of frozen human cortical bone, but compressive strength was diminished markedly among freeze-dried samples that had been irradiated with the same dose. Hamer et al[16] observed no effect on the elastic behavior of human cortical bone samples irradiated with 28 kGy, but there was a reduction in strength of approximately 64% when compared to non-irradiated samples. The same researchers[20] also suggested that deep-frozen human cortical bone irradiated with 30 kGy was less brittle than similar allografts irradiated at room temperature. Currey et al[15] confirmed that a "standard" radiation dose (29.5 kGy) administered at room temperature caused embrittlement of human cortical bone, thereby reducing its energy absorbing capacity. Indeed, Akkus and Rimnac[21] also demonstrated that it was easier to initiate and propagate a macrocrack in human cortical bone samples following 27.5 kGy of irradiation. Godette et al,[19] using a rabbit model, found significant reductions in torsional strength immediately following exposure to a 25 kGy dose in frozen tibial samples. Lastly, Randall et al[22] showed that a 30 kGy dose resulted in frequent microfractures and loss of torsional strength in rat femora and this effect was exacerbated among samples that had undergone freeze drying prior to irradiation.

A number of studies also have evaluated the impact of irradiation doses in the bactericidal range (i.e., < 20 kGy) on the static biomechanical properties of allogeneic cortical bone and have generally found the effects to be mild or negligible.[15-17, 23] For example, Komender[17] demonstrated no effect on the bending, torsional or compressive strength of human cortical bone samples irradiated with 5 or 10 kGy. Hamer et al[16] likewise observed only small decrements in the biomechanical properties of human cortical bone samples exposed to irradiation below 10 kGy administered at room temperature, with mild effects found at 16 kGy. Currey et al[15] found that a 17 kGy dose administered at room temperature reduced the energy absorbing capacity (e.g., work to fracture) of human cortical bone samples by about 50%, although the elastic properties of the bone remained unchanged. Finally, Simonian et al[23] demonstrated that the screw pullout strength in human tibial specimens was not affected by 16 to 19 kGy compared to non-irradiated control samples.

The results of studies evaluating the effects of irradiation on the biomechanical properties of allogeneic cancellous bone are more mixed. Cornu et al[24] confirmed that the addition of gamma irradiation at a dose of 25 kGy after freeze-drying of human femoral head samples resulted in a loss of their capacity to absorb energy in compression with resultant osseous tissue brittleness. In sharp contrast, Anderson et al[25] demonstrated that the compressive strength and elastic modulus of frozen, human cancellous bone specimens were unaffected by irradiation doses ranging from 10 kGy to 51 kGy; a dose of 60 kGy was required to cause significant tissue embrittlement. These findings were confirmed by Zhang et al[26] who demonstrated that the compressive strength of human iliac crest wedges was unaffected by 20 to 25 kGy of gamma irradiation administered in the frozen state. Irradiation with the same dose range in freeze-dried samples resulted in moderate, although not statistically significant, declines in tissue biomechanical properties.[26] Consequently, the published literature would suggest that an irradiation dose in the range of 20 to 25 kGy has a less demonstrable effect on the static biomechanical properties of allogeneic cancellous bone compared to cortical bone.

Connective Tissue Allografts: Since connective tissue allografts, such as tendons and ligaments, are composed primarily of type-I collagen, their biomechanical properties may be particularly susceptible to irradiation-associated denaturation such as tissue crimping.[27] A number of studies that have employed high doses (40–100 kGy) of irradiation to treat connective tissue allografts have uniformly reported marked

decreases in biomechanical properties, such as stiffness, rendering these tissues unacceptable for implantation.[28-31]

Irradiation of connective tissue allografts at lower doses has produced more encouraging results. For example, Smith et al[32] demonstrated that frozen porcine tendons exposed to 25 kGy showed no decrements in biomechanical properties compared to non-irradiated frozen grafts, whereas irradiation after freeze drying had a deleterious impact with tensile strength reduced by approximately 90%. Bettin et al[33] corroborated these findings by showing that 26 kGy administered to freeze-dried sheep ligaments significantly affected their maximum load to failure with somewhat lesser effects on graft stiffness. Similarly, Maeda et al[34] showed little effect on the tensile strength of canine tendons irradiated with 28 kGy followed by solvent drying. However, when solvent drying was performed as an initial processing step followed by the same dose of irradiation, tensile strength was only 39% of untreated control values.

Reducing the irradiation dose further to 20 kGy appears to assist in preserving the inherent biomechanical properties for connective tissue allografts. Fideler et al[29] found that a 20 kGy dose significantly reduced 4 of 7 biomechanical properties (maximum force, strain energy, modulus, maximum stress) tested in frozen, human patellar tendons compared to untreated controls but, importantly, graft stiffness was unaffected. Additionally, Haut and Powlison[35] and Gibbons et al[36] failed to demonstrate any noteworthy or statistically significant decrements in tendon biomechanical properties after irradiation with 20 kGy. Unfortunately, there have been no published studies, to date, examining the impact of lower doses (e.g., < 20 kGy) of irradiation on the static biomechanical properties of connective tissue allografts.

BIOLOGIC INCORPORATION AND CLINICAL PERFORMANCE OF IRRADIATED ALLOGRAFTS

Bone Allografts: Compared to the numerous studies evaluating the impact on static biomechanical properties, much less is known about the effects of irradiation treatment on the biologic incorporation and *in vivo* clinical performance of musculoskeletal allografts. Using a rat model, Jinno et al[37] demonstrated that a 15 kGy dose delivered immediately prior to aseptic tissue processing did not alter the histologic architecture or incorporation of allogeneic cortical bone grafts 4 or 6 months after implantation. In fact, after 6 months of implantation, irradiated processed allografts had significantly greater compressive strength than fresh syngeneic, fresh allogeneic and irradiated processed syngeneic grafts.[37] Similarly, Godette et al[19] noted little difference in biomechanical properties after 3 months in rabbit femoral segments irradiated with 25 kGy prior to implantation. This finding was in marked contrast to significant decrements in mechanical integrity found immediately after irradiation.[19]

While controlled human clinical trials are absent, irradiated bone allografts appear to offer satisfactory clinical performance similar to non-irradiated allografts.[38] In their seminal study, Basett and Packard[39] reported an approximate 85% clinical success rate among 1,037 patients receiving 1,759 cortical allografts irradiated with 20 kGy prior to implantation for a wide range of orthopaedic indications, including spinal fusions. Importantly, the infection rate in this patient population was less than 1%.[39] Similarly, another group of researchers reported a clinical success rate in excess of 90% after several years of followup in over 1,000 patients treated with freeze-dried, irradiated bone grafts for a variety of orthopaedic problems.[40, 41] Use of frozen, irradiated cancellous bone also resulted in 33 of 35 (94%) patients showing clinical success after acetabular reconstructive procedures.[42] Finally, Lietman et al[43] reported a significantly higher fracture rate between irradiated (10–30 kGy) (39%) and non-irradiated (18%) massive structural allografts with a mean followup of 5 years. However, the frequency of nonunion (7% vs. 19%) and the incidence of postoperative infection (0% vs. 11%) favored patients receiving irradiated grafts.[43]

Special mention should be made regarding bone allografts that have undergone demineralization as a means of providing an osteoinductive matrix for various bone grafting procedures.[6] It has been shown that irradiation can have a deleterious effect on the inherent growth factors responsible for the inductive potential of demineralized bone matrix particularly with doses in excess of 20 kGy.[44-46] Several studies demonstrated that a 25 kGy dose of irradiation administered after the demineralization process reduced the osteoinductivity of the matrix by up to 50%,[47, 48] but histologic and morphometric graft characteristics and biologic incorporation were generally unaffected.[48, 49] Interestingly, 2 studies showed that even irradiation doses greater than 30 kGy do not extinguish the osteoinductive potential of demineralized bone matrix if the treatment is performed in frozen samples.[50, 51] To the contrary, studies of the effects of irradiation in freeze-dried demineralized samples suggest that even fairly low doses (e.g., 15 kGy) can extinguish osteoinductivity.[50, 52] Finally, it should be noted that to retain osteoinductivity, bone samples should always be irradiated after demineralization as pre-processing irradiation of undemineralized bone significantly reduces the bone forming potential of the resultant matrix.[48]

Connective Tissue Allografts: Limited data are available regarding the biologic incorporation of irradiated connective tissue allografts; nonetheless, results have been promising. Goertzen et al[53, 54] reported that canine patellar tendon-bone allografts treated with 20 kGy in argon gas protection compared favorably with non-irradiated grafts with respect to maximum load to failure 12 months after implantation. Additionally, Maeda et al[55] observed that rat tendons irradiated with 28 kGy had strikingly similar tensile strength to fresh frozen controls by 1 month after implantation and this effect was maintained through 2 years of followup. These results were in sharp contrast to marked deficits in tensile strength observed immediately following irradiation.[55] Mae et al[56] confirmed that treatment of rat patellar tendons with a dose of 25 kGy resulted in a significant reduction in tensile strength prior to implantation compared to non-irradiated grafts. However, the difference in biomechanical properties between irradiated and non-irradiated grafts disappeared as early as 1 month after implantation, with equivalency in tensile strength between grafts maintained through 6 months of followup.

INTERPRETATION, CONCLUSIONS AND RECOMMENDATIONS

Musculoskeletal allograft usage among orthopedic and neurosurgeons has risen dramatically over the past two decades.[2] Bone pieces in various shapes, sizes and configurations as well as allogeneic matrices are now available to surgeons, significantly enhancing their ability to perform many complex reconstructive procedures with greater assurance of a clinically successful outcome.[2] In addition to the well documented complications associated with harvesting autologous tissue, many patients simply do not have an adequate supply of autograft for these procedures.[7] Connective tissue allografts have likewise enjoyed more widespread usage in recent years as numerous studies have demonstrated that allografts show comparable results to autologous tissue for ACL reconstruction in particular.[4] Using allogeneic tendon material for ACL reconstruction offers the advantage of obviating donor site morbidity, reducing operative time, eliminating restrictions on graft size, and possibly lowering the incidence of arthrofibrosis.[4] In general, allograft tissues are extremely safe and complications resulting from disease transmission are extremely rare. However, cases of allograft contamination have been reported and the consequences can be catastrophic for the patient.[1] Therefore, strict adherence to standardized procurement and processing guidelines and protocols is mandatory.[1, 4]

The goal of allograft tissue processing is to provide the safest possible material to the surgeon while preserving the inherent tissue characteristics of the graft. Even with adequate donor screening and aseptic tissue processing, there remains a low risk of allograft contamination. Consequently, tissue banks routinely irradiate allografts to offer a further level of assurance that grafts are safe and, indeed, low levels of irradiation provide sufficient bactericidal coverage and act to attenuate immunogenicity.[2, 11] A standard dose of 25 kGy is routinely used to treat allografts.[4] However, there appears to be little scientific basis for this dose level as it is clearly greater than the dose required to provide bactericidal coverage in the setting of aseptic tissue processing.[15]

While there are some reports to the contrary, on balance, usage of the currently recommended irradiation dose of 25 kGy appears to moderately impact the static biomechanical properties of both bone and connective tissue allografts and this effect is likely exacerbated among specimens that have been freeze-dried and irradiated at room temperature. These effects appear to be less demonstrable for doses in the bactericidal range, i.e., 10–20 kGy. While it is not entirely clear whether moderate alterations to the biomechanical properties of allografts will substantially effect ultimate graft performance, use of lower doses of irradiation (e.g., 15 kGy) avoids the potential risk of tissue damage while maintaining bactericidal coverage. Clearly, for some applications that serve a functional purpose such as the use of patellar tendons for ACL reconstruction, there is a demand for preservation of tissue biomechanical integrity that necessitates use of the lowest practical irradiation dose. In contrast, other applications such as the use of cancellous bone chips employed in a graft composite to support acetabular reconstructions, are undoubtedly less sensitive to biomechanical property alterations resulting from irradiation, making the exact dose level less relevant.

It has been almost uniformly demonstrated that excessively high irradiation doses capable of providing complete tissue sterility are sufficiently high to cause irreparable tissue damage to allografts.[15] Consequently, doses of 40 kGy or greater cannot be recommended for processing allogeneic tissue.

The adoption of stringent screening procedures by most reputable tissue banks is undoubtedly, in large part, responsible for averting viral transmission in particular. Indeed, the current process includes a detailed medical and social history, serologic tests for HIV I/II antibodies, HIV antigen, polymerase chain reaction HIV, hepatitis B surface antigen, hepatitis B surface antibodies, hepatitis B core antibodies, and hepatitis C antibodies.[1] Optionally, a lymph node analysis of donors is included.[1] Clearly, it is more efficient to screen donor samples for viral contamination than to rely on processing techniques such as irradiation to provide effective tissue sterility.

The temperature at which irradiation is used in allograft processing appears to be crucial. Most studies show that administering irradiation at room temperature in freeze dried samples is particularly deleterious to the biomechanical properties of the allograft and this practice should be avoided. Thus, it is preferable to irradiate tissue in the frozen state. This guidance also applies to allogeneic demineralized bone matrix, with the caveat that irradiation should always be administered to already demineralized tissue prior to storage.

In conclusion, an irradiation dose in the range of 10 to 15 kGy is recommended to treat musculoskeletal allografts prior to distribution and transplantation. This dose provides effective bactericidal coverage and very likely has minimal impact on the inherent biomechanical properties of the allograft. This dose also may offer the additional benefit of providing a graft that is less antigenic and more likely to offer good biologic incorporation and integration. All musculoskeletal allografts should undergo rigorous donor screening and serologic testing prior to aseptic processing.

REFERENCES

1. Barbour SA, King W. Basic science update. The safe and effective use of allograft tissue-an update. *Am J Sports Med.* 2003;31:791-797.

2. Boyce T, Edwards J, Scarborough N. Allograft bone. The influence of processing on safety and performance. *Orthop Clin North Am.* 1999;30:571-581.

3. Tomford WW, Mankin HJ. Bone banking. Update on methods and materials. *Orthop Clin North Am.* 1999;30:565-570.

4. Vangsness CT, Jr., Garcia IA, Mills CR, Kainer MA, Roberts MR, Moore TM. Allograft transplantation in the knee: tissue regulation, procurement, processing, and sterilization. *Am J Sports Med.* 2003;31:474-481.

5. Stevenson S. Biology of bone grafts. *Orthop Clin North Am.* 1999;30:543-552.

6. Russell JL, Block JE. Clinical utility of demineralized bone matrix for osseous defects, arthrodesis, and reconstruction: impact of processing techniques and study methodology. *Orthopedics.* 1999;22:524-531.

7. Russell JL, Block JE. Surgical harvesting of bone graft from the ilium: point of view. *Med Hypotheses.* 2000;55:474-479.

8. Angermann P, Jepsen OB. Procurement, banking and decontamination of bone and collagenous tissue allografts: guidelines for infection control. *J Hosp Infect.* 1991;17:159-169.

9. Bright RW, Smarsh JD, Gambill VM. *Osteochondral Allografts: Biology, banking and clinical applications.* Vol 1. 1st ed. Boston/Toronto: Little, Brown and Company; 1983.

10. Pelker RR, Friedlaender GE, Markham TC. Biomechanical properties of bone allografts. *Clin Orthop.* 1983:174;54-57.

11. Vangsness CT, Jr., Triffon MJ, Joyce MJ, Moore TM. Soft tissue for allograft reconstruction of the human knee: a survey of the American Association of Tissue Banks. *Am J Sports Med.* 1996;24:230-234.

12. Turner TC, Basset CAL, Pate JW, Sawyer PN. Sterilization of preserved bone grafts by high-voltage cathode irradation. *J Bone Joint Surg Am.* 1956;38:862-884.

13. Cohen J. Cathode ray sterilization of bone grafts. *Arch Surg.* 1955;71:784-789.

14. DeVries PH, Badgley CE, Hartman JT. Radiation sterilization of homologous bone transplants utilizing radioactive cobalt. *J Bone Joint Surg Am.* 1958;40:187-203.

15. Currey JD, Foreman J, Laketic I, Mitchell J, Pegg DE, Reilly GC. Effects of ionizing radiation on the mechanical properties of human bone. *J Orthop Res.* 1997;15:111-117.

16. Hamer AJ, Strachan JR, Black MM, Ibbotson CJ, Stockley I, Elson RA. Biochemical properties of cortical allograft bone using a new method of bone strength measurement. A comparison of fresh, fresh-frozen and irradiated bone. *J Bone Joint Surg Br.* 1996;78:363-368.

17. Komender A. Influence of preservation on some mechanical properties of human haversian bone. *Mater Med Pol.* 1976;8:13-17.

18. Triantafyllou N, Sotiropoulos E, Triantafyllou JN. The mechanical properties of the lyophylized and irradiated bone grafts. *Acta Orthop Belg.* 1975;41:35-44.

19. Godette GA, Kopta JA, Egle DM. Biomechanical effects of gamma irradiation on fresh frozen allografts in vivo. *Orthopedics.* 1996;19:649-653.

20. Hamer AJ, Stockley I, Elson RA. Changes in allograft bone irradiated at different temperatures. *J Bone Joint Surg Br.* 1999;81:342-344.

21. Akkus O, Rimnac CM. Fracture resistance of gamma radiation sterilized cortical bone allografts. *J Orthop Res.* 2001;19:927-934.

22. Randall RL, Pelker RR, Friedlaender GE, Goldsmith SL, Panjabi MM. Sequential dependence of freeze-drying and irradiation on biomechanical properties of rat bone. *Am J Orthop.* 2002;31:129-134.

23. Simonian PT, Conrad EU, Chapman JR, Harrington RM, Chansky HA. Effect of sterilization and storage treatments on screw pullout strength in human allograft bone. *Clin Orthop.* 1994:302;290-296.

24. Cornu O, Banse X, Docquier PL, Luyckx S, Delloye C. Effect of freeze-drying and gamma irradiation on the mechanical properties of human cancellous bone. *J Orthop Res.* 2000;18:426-431.

25. Anderson MJ, Keyak JH, Skinner HB. Compressive mechanical properties of human cancellous bone after gamma irradiation. *J Bone Joint Surg Am.* 1992;74:747-752.

26. Zhang Y, Homsi D, Gates K, Oakes K, Sutherland V, Wolfinbarger L, Jr. A comprehensive study of physical parameters, biomechanical properties, and statistical correlations of iliac crest bone wedges used in spinal fusion surgery. IV. Effect of gamma irradiation on mechanical and material properties. *Spine.* 1994;19:304-308.

27. Belkoff SM, Haut RC. Microstructurally based model analysis of gamma-irradiated tendon allografts. *J Orthop Res.* 1992;10:461-464.

28. Donald PJ, Deckard-Janatpour K, Sharkey N, Lagunas-Solar M. The effects of irradiation dose on the stiffness of cartilage grafts. *Ann Plast Surg.* 1996;36:297-303.

29. Fideler BM, Vangsness CT, Jr., Lu B, Orlando C, Moore T. Gamma irradiation: effects on biomechanical properties of human bone- patellar tendon-bone allografts. *Am J Sports Med.* 1995;23:643-646.

30. Rasmussen TJ, Feder SM, Butler DL, Noyes FR. The effects of 4 Mrad of gamma irradiation on the initial mechanical properties of bone-patellar tendon-bone grafts. *Arthroscopy.* 1994;10:188-197.

31. Salehpour A, Butler DL, Proch FS, et al. Dose-dependent response of gamma irradiation on mechanical properties and related biochemical composition of goat bone-patellar tendon-bone allografts. *J Orthop Res.* 1995;13:898-906.

32. Smith CW, Young IS, Kearney JN. Mechanical properties of tendons: changes with sterilization and preservation. *J Biomech Eng.* 1996;118:56-61.

33. Bettin D, Rullkotter V, Polster J, Fuchs S. Primary biomechanical influence of different sterilization methods on a freeze-dried bone-ligament transplant. *Arch Orthop Trauma Surg.* 1999;119:236-240.

34. Maeda A, Inoue M, Shino K, et al. Effects of solvent preservation with or without gamma irradiation on the material properties of canine tendon allografts. *J Orthop Res.* 1993;11:181-189.

35. Haut RC, Powlison AC. The effects of test environment and cyclic stretching on the failure properties of human patellar tendons. *J Orthop Res.* 1990;8:532-540.

36. Gibbons MJ, Butler DL, Grood ES, Bylski-Austrow DI, Levy MS, Noyes FR. Effects of gamma irradiation on the initial mechanical and material properties of goat bone-patellar tendon-bone allografts. *J Orthop Res.* 1991;9:209-218.

37. Jinno T, Miric A, Feighan J, Kirk SK, Davy DT, Stevenson S. The effects of processing and low dose irradiation on cortical bone grafts. *Clin Orthop.* 2000:375;275-285.

38. Loty B, Courpied JP, Tomeno B, Postel M, Forest M, Abelanet R. Bone allografts sterilised by irradiation. Biological properties, procurement and results of 150 massive allografts. *Int Orthop.* 1990;14:237-242.

39. Basset CAL, Packard SD. A clinical assay of cathode ray sterilized cadaver bone grafts. *Acta Orthop Scand.* 1959;28:198-211.

40. Komender J, Malczewska H, Komender A. Therapeutic effects of transplantation of lyophilized and radiation- sterilized, allogeneic bone. *Clin Orthop.* 1991:272;38-49.

41. Marczynski W, Tylman D, Komender J. Long-term follow up after transplantation of frozen and radiation sterilized bone grafts. *Ann Transplant.* 1997;2:64-66.

42. Kwiatkowski K, Ratynski G. The usage of frozen allografts of the spongy bone in filling the loss of bone after loosening of the hip prosthesis. *Ann Transplant.* 1999;4:59-63.

43. Lietman SA, Tomford WW, Gebhardt MC, Springfield DS, Mankin HJ. Complications of irradiated allografts in orthopaedic tumor surgery. *Clin Orthop.* 2000:375;214-217.

44. Ijiri S, Yamamuro T, Nakamura T, Kotani S, Notoya K. Effect of sterilization on bone morphogenetic protein. *J Orthop Res*. 1994;12:628-636.

45. Puolakkainen PA, Ranchalis JE, Strong DM, Twardzik DR. The effect of sterilization on transforming growth factor beta isolated from demineralized human bone. *Transfusion.* 1993;33:679-685.

46. Urist MR, Hernandez A. Excitation transfer in bone. Deleterious effects of cobalt 60 radiation- sterilization of bank bone. *Arch Surg.* 1974;109:586-593.

47. Munting E, Wilmart JF, Wijne A, Hennebert P, Delloye C. Effect of sterilization on osteoinduction. Comparison of five methods in demineralized rat bone. *Acta Orthop Scand.* 1988;59:34-38.

48. Schwarz N, Redl H, Schiesser A, et al. Irradiation-sterilization of rat bone matrix gelatin. *Acta Orthop Scand.* 1988;59:165-167.

49. Hallfeldt KK, Stutzle H, Puhlmann M, Kessler S, Schweiberer L. Sterilization of partially demineralized bone matrix: the effects of different sterilization techniques on osteogenetic properties. *J Surg Res.* 1995;59:614-620.

50. Dziedzic-Goclawska A, Ostrowski K, Stachowicz W, Michalik J, Grzesik W. Effect of radiation sterilization on the osteoinductive properties and the rate of remodeling of bone implants preserved by lyophilization and deep-freezing. *Clin Orthop.* 1991:272;30-37.

51. Wientroub S, Reddi AH. Influence of irradiation on the osteoinductive potential of demineralized bone matrix. *Calcif Tissue Int.* 1988;42:255-260.

52. Buring K, Urist MR. Effects of ionizing radiation on the bone induction principle in the matrix of bone implants. *Clin Orthop*. 1967:55;225-234.

53. Goertzen MJ, Clahsen H, Schulitz KP. Anterior cruciate ligament reconstruction using cryopreserved irradiated bone-ACL-bone-allograft transplants. *Knee Surg Sports Traumatol Arthrosc.* 1994;2:150-157.

54. Goertzen MJ, Clahsen H, Burrig KF, Schulitz KP. Sterilisation of canine anterior cruciate allografts by gamma irradiation in argon. Mechanical and neurohistological properties retained one year after transplantation. *J Bone Joint Surg Br.* 1995;77:205-212.

55. Maeda A, Horibe S, Matsumoto N, Nakamura N, Mae T, Shino K. Solvent-dried and gamma-irradiated tendon allografts in rats. Mechanical and morphological evaluation. *J Bone Joint Surg Br.* 1998;80:731-736.

56. Mae T, Shino K, Maeda A, Toritsuka Y, Horibe S, Ochi T. Effect of gamma irradiation on remodeling process of tendon allograft. *Clin Orthop.* 2003:414;305-314.

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