Acta Orthop. Belg., 2011, **77**, 670-675

ORIGINAL STUDY

Mechanical strength of cortical allografts with gamma radiation versus ethylene oxide sterilization

Zongke ZHOU, Tingwu QIN, Jing YANG, Bin SHEN, Pengde KANG, Fuxing PEI

From West China Hospital of Sichuan University, Chengdu, China

We investigated the effects of gamma irradiation versus ethylene oxide (ETO) sterilization on the mechanical strength of cortical bone grafts. Tibias were collected from cadavers of mature goats. Sixty test specimens were randomized into four groups : fresh (no processing), frozen (freezing at –70°C), gamma-irradiated, and ETO-sterilized specimens. Torsion, three-point bending, and compression testing were separately performed with a material testing machine. Parameters studied included maximum stress, strain, deflection, extension, load, shear modulus, and E-modulus. Compared with findings for the fresh specimens, findings were as follows for gamma-irradiated specimens : maximal shear modulus, reduced by 48% ; shear stress, by 55% ; deflection, by 71% ; bending stress, by 51% ; bending strain, by 74% ; extension, by 60% ; and compression strain, by 50%. However, there were no reductions in those parameters for the frozen specimens or the ETO-sterilized specimens. These findings confirm that shear, bending, and compression strength of cortical allografts are weakened by gamma irradiation at room temperature. To maintain optimum mechanical properties, ETO sterilization of allografts is better than gamma sterilization, especially for cortical bone, because it is usually used in load-bearing settings.

Keywords : allografts ; biomechanical strength ; cortical bone ; deep-frozen ; sterilization methods.

INTRODUCTION

Bone allografts are increasingly being used in orthopaedic surgery for both structural and morselization purposes. One of the major concerns with allografts is the risk of infection and disease transmission (*20*). Therefore, to maintain maximum safety, some forms of tissue disinfection are necessary to protect recipients from virus transmission by infected allografts. Currently, the most commonly used methods for bactericidal and viricidal purposes are irradiation (both beta and gamma) and ethylene oxide (ETO) sterilization (*3,7,8,15*).

One concern associated with gamma radiation is the alteration of the mechanical properties of the allografts. Previous studies showed that gamma

■ Zongke Zhou, MD, PhD, Orthopaedic Surgeon, Associate Professor of Orthopaedic Surgery.
■ Jing Yang, MD, Orthopaedic Surgeon, Associate Professor of Orthopaedic Surgery.
■ Bin Shen, MD, PhD, Orthopaedic Surgeon, Professor of Orthopaedic Surgery.
■ Pengde Kang, MD, PhD, Orthopaedic Surgeon, Associate Professor of Orthopaedic Surgery.
■ Fuxing Pei, MD, Orthopaedic Surgeon, Professor of Orthopaedic Surgery.
Department of Orthopaedic Surgery, West China Hospital of Sichuan University, Chengdu, China.
■ Tingwu Qin, PhD, Scientist, Professor of Biomechanics.
Stem Cell and Tissue Engineer Laboratory, West China Hospital of Sichuan University, Chengdu, China.
Correspondence : Dr. Fuxing Pei, Department of Orthopaedic Surgery, West China Hospital of Sichuan University, No. 37 Guo Xue Xiang, Chengdu 610041, China.
E-mail : zhouzongke@scu.edu.cn

No benefits or funds were received in support of this study

irradiation significantly reduced the resistance of cortical bone to fatigue crack growth (*19*), and the damage is induced via free-radical attack on the collagen (*2*). One study showed that it is easier to initiate and propagate a macrocrack from stress concentration due to the inhibition of damage formation at and near the crack tip for cortical bone irradiated at 27.5 kGy (*3*). However, the irradiation dose required to inactivate the bioburden of human immunodeficiency virus in allograft bone is 35 kGy, and furthermore, the irradiation dose required to achieve a sterility assurance level of 10^{-6} is 89 kGy. This dose exceeds current recommendations for sterilizing medical products and the current practice of many bone banks (*6*). It is difficult to choose between mechanical properties and virus inactivation for bone allografts sterilized by gamma irradiation.

In clinical practice, cortical bone grafts usually are used in load-bearing settings such as skeletal reconstruction, spinal fusion, and tumour surgery (*17*). The mechanical properties of transplanted bone grafts inevitably determine the short-term and midterm results of the procedure performed ; therefore, adequate mechanical performance of the grafts is required.

Biomechanical studies of soft tissues, mostly patellar bone-tendon-bone preparations, have quantified irradiation damage (*11*), but no clinical alteration of tissue function has been shown with ETO disinfection (*5*). ETO, a strong alkylator, is a highly effective virucide (*16,22*). Bone allografts sterilized with terminal ETO disinfection are used in the field of orthopedic surgery.

There is limited documentation of whether ETO sterilization impairs the mechanical properties of bone. Thus, we investigated the effects of sterilization methods on the mechanical properties of deep-frozen bone to select a sterilization method for cortical bone grafts.

MATERIALS AND METHODS

Preparation of Test Samples

Tibias were collected from cadavers of 12-month-old mature goats weighing 16 to 18 kg. Bone quality was checked by observation. The middle section of each tibia was selected for the test samples. Each section was cut into four pieces through the middle coronal plane and sagittal plane, using a hacksaw, to ready it for a torsion test and a three-point bending test. Also, some of the sections were cut into hemi-cylinders through the middle coronal plane for a compression test. Using a grinder, we machined the samples into rectangular parallelepipeds that were 40 mm long, 5 mm wide, and 2 mm thick for torsion and three-point bending tests and parallelepipeds that were 20 mm long, 10 mm wide, and 2 mm thick for compression tests.

Grouping and Processing Methods

Sixty test pieces were randomized into four groups : fresh, frozen, gamma-irradiated, and ETO-sterilized. The fresh and frozen test pieces were stored at 4°C and –70°C, respectively, for one month. The gamma-group test pieces were irradiated for nine hours with a dose of 25 kGy by gamma ray at room temperature and noninert gas immediately after processing, and then stored at –70°C for one month. A Fricke dosimeter was used to record the radiation dose. The ETO-group samples were sterilized for 16 hours at 48°C using a Steri-Vac (3M Health Care, St. Paul, MN, USA) hospital ETO sterilization chamber immediately after processing, and then stored at –70°C for one month.

Mechanical Test

The torsion test, three-point bending test, and compression test were performed separately for each group (n = 5 pieces for each group). The test pieces were packaged without any additives in sterile, airtight, triple-water-resistant plastic packages until it was time to conduct each test. All samples were first transferred to a refrigerator at 4°C 24 hours before testing, and then rehydrated in saline for 2 hours. The mechanical testing of each specimen was performed using an Instron 8874 material test machine (Instron Co., Norwood, MA, USA), at room temperature, with a relative humidity of 50% to 75%.

Torsion Test

There was no axial loading for the samples undergoing torsion testing, and the maximal torsion angle was between 25° and 90°. The torsion was set at one cycle per second. A computer automatically recorded torque

Table I. — Constant depending on the ratio of width to thickness

h/b	1.0	1.2	1.5	1.75	2.0	2.5	3.0	4.0
α	0.208	0.219	0.231	0.239	0.246	0.258	0.267	0.282
β	0.141	0.166	0.196	0.214	0.229	0.249	0.293	0.281

b, sample thickness ; h, sample width.

(T) and torsion angle (ψ) during testing. The shear modulus (G), maximal shear stress (τ_{max}), and maximal shear strain (γ) were calculated according to the following equation at the maximal torsion angle for each sample :

$$G = TL/\psi\beta hb3 \ \tau_{max} = T/\alpha hb2 \ \gamma = \tau_{max}/G$$

where *L* is sample length, *h* is sample width, *b* is sample thickness, and *α* and *β* are constants depending on the ratio of width to thickness (Table I).

Three-Point Bending Test

The span of the each test was 30 mm, and the loading point was set on the inner surface of all samples. A computer automatically recorded data for maximal bending load and deflection of the testing samples. The maximal bending stress, maximal bending strain, maximal deflection, and bending modulus were obtained from the testing data.

Compression Test

A compression load was applied at a rate of 5 mm/min, and the loading direction was from the proximal to the distal end of the diaphysis. Testing was stopped when the maximal load was reduced to 50% because of sample fracture or breakage. A computer recorded data on maximal compression load and displacement. The maximal compression stress and strain were calculated on the basis of the testing data.

Statistics

All data were expressed as mean (SD), and statistical analyses were done using SPSS for Windows (version 13.0 ; SPSS Inc., Chicago, IL, USA). We considered p values < 0.05 to be significant. One-way analysis of variance was used to evaluate differences between groups regarding mechanical properties.

RESULTS

Sample Damage after Testing

Spiral fractures occurred in the samples used for torsion testing in the fresh, frozen, and ETO groups ; however, comminuted fractures occurred in the gamma group. All samples for the three-point bending test broke in the middle in all four groups. After the compression test, all samples in all four groups had compression fractures at both ends.

Outcome of Mechanical Tests

Torsion Test

Compared with the fresh group, the gamma group's maximal shear modulus and maximal shear stress were reduced by 48% ($p < 0.01$) and by 55% ($p < 0.01$), respectively. The maximal shear modulus was reduced by 10% in the frozen and ETO groups, and the maximal shear stress was reduced by 12% in the frozen group and by 18% in ETO group. However, the differences were not significant (Table II).

Three-Point Bending Test

The maximal bending loading was decreased by 55% ($p < 0.01$), the deflection was decreased by 71% ($p < 0.01$), maximal bending stress was reduced by 51% ($p < 0.01$), and the maximal strain was reduced by 74% ($p < 0.01$) in the gamma group compared with the fresh group. However, there were no significant differences among the fresh, frozen, and ETO groups (Table II).

Compression Test

Compared with the fresh group, the gamma group's extension was decreased by 60% ($p < 0.05$) and maximal compression strain was reduced by 50% ($p < 0.01$). There were no significance differences for extension and maximal compression strain among the frozen, ETO, and fresh groups. There were also no significant differences among the four groups regarding maximal compression loading and stress (Table II).

Table II. — Summary of results, reported as mean (SD)

Values	Fresh	Frozen	Gamma	ETO
Torsion test				
Shear stress (MPa)	109.7 (28.8)	96.1 (21.6)	49.3 (13.6)	89.6 (7.21)
Shear strain (%)	7.02 (0.77)	6.99 (0.54)	6.12 (1.57)	7.07 (1.48)
Shear modulus (GPa)	1.56 (0.31)	1.39 (0.40)	0.81 (0.12)	1.36 (0.31)
Three points bending test				
Maximum load (N)	139 (10.0)	130 (13.5)	63 (7.6)	135 (9.6)
Deflection (mm)	2.2 (0.3)	2.4 (0.4)	0.6 (0.1)	2.2 (0.1)
Flexure stress (MPa)	239 (33.3)	229 (19.2)	118 (18.5)	220 (31.1)
Flexure strain (%)	3.3 (0.25)	3.9 (0.19)	0.8 (0.13)	3.8 (0.22)
E-modulus (Gpa)	21.4 (3.02)	17.6 (1.69)	18.3 (4.17)	17.6 (1.41)
Compression test				
Maximum load (kN)	2.58 (0.13)	2.48 (0.21)	2.28 (0.13)	2.30 (0.35)
Compression extension (mm)	1.14 (0.59)	1.06 (0.53)	0.46 (0.15)	1.11 (0.31)
Maximum stress (MPa)	45.8 (5.22)	43.3 (7.87)	46.0 (1.57)	43.7 (2.00)
Compression strain (%)	4.68 (1.48)	4.48 (0.41)	2.33 (0.88)	4.61 (0.96)

ETO, ethylene oxide.

DISCUSSION

Maintenance of biomechanical integrity after terminal processing or sterilization is vital for the successful use of cortical bone allografts in orthopaedics, because cortical allografts are used to restore skeletal integrity, as in reconstruction in large-bone defects and spinal fusion, often under weight-bearing, shear, and flexure conditions. The mechanical strength of the graft is crucial.

Previous studies have reported that cortical bone becomes brittle because of gamma-radiation sterilization (*19*), which increases microfractures (*10*). During gamma irradiation, highly reactive hydroxyl radicals are formed because of ionization of water molecules. These free radicals have been speculated to impair the integrity of collagen molecules (*12*). Free-radical damage to collagen extracted from tendons was previously inhibited by using the scavengers thiourea and cysteamine (*18*). In one study, there was a several-fold improvement in the mechanical properties of specimens sterilized in the presence of thiourea in comparison with those irradiated in its absence (*1*).

We investigated the effect of deep-freezing on the mechanical strength of cortical bone allografts compared with unprocessed fresh bone, as well as the effect of two commonly used sterilization methods (gamma irradiation and ETO), each combined with deep-freezing, evaluated by failure during shear, bending, or axial compression.

The effect on biomechanics of gamma irradiation of allograft bone is dose dependent, and higher doses of gamma radiation will cause greater losses in the biomechanical integrity of the tissue (*8*). Findings from a study by Balsley *et al* support the use of low-dose (18.3-21.8 kGy) and moderate-dose (24.0-28.5 kGy) gamma irradiation to sterilize bone grafts (*4*). The standard dose range for sterilization of bone grafts is 25 to 35 kGy ; thus, the level of radiation in our study was 25 kGy.

We found that in the gamma group, maximal shear modulus, shear stress, deflection, bending stress, and bending strain were reduced by 48%, 55%, 71%, 51%, and 74%, respectively. In comparison with the fresh group, the gamma group showed reductions in extension and compression strain of 60% and 50%, respectively. These results

imply that shear, bending, and compression strength of cortical allografts are weakened by gamma irradiation.

Conversely, fewer changes in biomechanics have been observed with irradiation under dry ice (*9*) and while the bone is frozen (*12*). Two reports by Cornu *et al* noted that the temperature during irradiation plays an important role in mechanics loss in bone grafts (*8,9*). Freeze-drying induced a 19% reduction in strength and a 20% reduction in stiffness, whereas work to failure was not significantly modified. Adding gamma irradiation at room temperature to the sequence induced a 42% reduction in strength, a 21% reduction in stiffness, and, most concerning of all, a 75% reduction in work to failure (*8*). In our study, irradiation of frozen bone (25-kGy dose) did not cause any significant reduction in ultimate strength, stiffness, or work to failure. The addition of the freeze-drying process before or after irradiation resulted in a mean drop of 35% and 31% in ultimate strength, 14% and 37% in stiffness, and 46% and 37% in work to failure. Unlike irradiation at room temperature, irradiation under dry ice of bone treated with solvent detergent seemed to have no detrimental effect on the mechanical properties of cancellous bone (*9*).

Hamer *et al* reported that bone irradiated at –78°C was less brittle and had less collagen damage than when irradiated at room temperature, because freezing reduces the mobility of water molecules and may therefore decrease the production of free radicals that can destroy collagen alpha chains (*12*). A limitation of our study is that irradiation was done at room temperature, which acted synergistically with gamma radiation to cause mechanical loss in the cortical bone grafts.

There have been only two studies of whether ETO sterilization weakens the mechanical properties of allografts, and both concluded that it does not do so. In the first study, Simonian *et al* sterilized allograft with ETO, and then performed screw pullout tests using four 3.5-mm cortical screws per segment, finding that screw pullout force was undiminished (*21*). The second study, by Hofmann *et al*, confirmed that the bending strength and shear strength of cortical pins were not decreased after sterilization with ETO (*13*).

We found that ETO sterilization had no significant effects on the shear strength, bending strength, or compression strength of cortical bone. From the standpoint of preservation of mechanical properties, ETO sterilization of allografts is better than gamma sterilization, especially for cortical bone. This is very important because cortical allograft is usually used in load-bearing settings. In the clinical setting, ETO exposure at 40°C is recommended as a true sterilization procedure because of its negligible effect on osteoinductive capacity (*23*) ; moreover, the reduction of residual ETO is an important clinical issue. A combination of repeated preoperative aeration, more than 2 weeks' storage before use, and intraoperative rinsing with 500 mL of saline for 10 minutes can effectively reduce the amount of residual ETO in freeze-dried bone allografts (*14*).

Twenty-five kGy of gamma radiation at room temperature reduces the shear strength, bending strength, and compression strength of cortical bone, yet ETO sterilization has no significant effects on any bone properties. To preserve mechanical properties, ETO sterilization of allografts is better than gamma sterilization, especially for cortical bone. This is critical because cortical bone grafts are usually used in load-bearing settings.

Acknowledgments

Our institution received funding in support of this study from the National High Technology Research and Development Program of China (863 Program, grant 2006AA02A135), Key Projects in the National Science & Technology Pillar Program During the Eleventh Five-Year Plan Period (grant 2006BAI16B01), and PhD Programs Foundation of Ministry of Education of China (grant 20070610150).

REFERENCES

1. **Akkus O, Belaney RM.** Sterilization by gamma radiation impairs the tensile fatigue life of cortical bone by two orders of magnitude. *J Orthop Res* 2005 ; 23 : 1054-1058.
2. **Akkus O, Belaney RM, Das P.** Free radical scavenging alleviates the biomechanical impairment of gamma radiation sterilized bone tissue. *J Orthop Res* 2005 ; 23 : 838-845.
3. **Akkus O, Rimnac CM.** Fracture resistance of gamma radiation sterilized cortical bone allografts. *J Orthop Res* 2001 ; 19 : 927-934.
4. **Balsly CR, Cotter AT, Williams LA *et al*.** Effect of low dose and moderate dose gamma irradiation on the

mechanical properties of bone and soft tissue allografts. *Cell Tissue Banking* 2008 ; 9 : 289-298.

5. **Bechtold JE, Eastlund DT, Butts MK *et al*.** The effects of freeze-drying and ethylene oxide sterilization on the mechanical properties of human patellar tendon. *Am J Sports Med* 1994 ; 22 : 562-566.
6. **Campbell DG, Li P.** Sterilization of HIV with irradiation : relevance to infected bone allografts. *Aust N Z J Surg* 1999 ; 69 : 517-521.
7. **Campbell DG, Li P, Stephenson AJ, Oakeshott RD.** Sterilization of HIV by gamma irradiation : a bone allograft model. *Int Orthop* 1994 ; 18 : 172-176.
8. **Cornu O, Banse X, Docquier PL *et al*.** Effect of freeze drying and gamma irradiation on the mechanical properties of human cancellous bone. *J Orthop Res* 2000 ; 18 : 426-431.
9. **Cornu O, Boquet J, Nonclercq O *et al*.** Synergetic effect of freeze-drying and gamma irradiation on the mechanical properties of human cancellous bone. *Cell Tissue Bank* 2010 Aug 12. DOI 10.1007/s10561-010-9209-1. Epub ahead of print.
10. **Dux SJ, Ramsey D, Chu EH *et al*.** Alterations in damage processes in dense cancellous bone following gamma-radiation sterilization. *J Biomech* 2010 ; 43 : 1509-1513.
11. **Fideler BM, Vangsness CT Jr, Lu B *et al*.** Gamma irradiation : effects on biomechanical properties of human bone-patellar tendon-bone allografts. *Am J Sports Med* 1995 ; 23 : 643-646.
12. **Hamer AJ, Stockley I, Elson RA.** Changes in allograft bone irradiated at different temperatures. *J Bone Joint Surg* 1999 ; 81B : 342-344.
13. **Hofmann C, Schadel-Hopfner M, Berns T *et al*.** [Influence of processing and sterilization on the mechanical properties of pins made from bovine cortical bone.] (in German). *Unfallchirurg* 2003 ; 106 : 478-482.
14. **Kaku N, Tsumura H, Kataoka M *et al*.** Influence of aeration, storage, and rinsing conditions on residual ethylene oxide in freeze-dried bone allograft. *J Orthop Sci* 2002 ; 7 : 238-242.
15. **Kearney JN, Bojar R, Holland KT.** Ethylene oxide sterilisation of allogenic bone implants. *Clin Mater* 1993 ; 12 : 129-135.
16. **Maillard JY, Russell AD.** Viricidal activity and mechanisms of action of biocides. *Sci Prog* 1997 ; 80 (Pt 4) : 287-315.
17. **Matejovsky Z Jr, Matejovsky Z, Kofranek I.** Massive allografts in tumour surgery. *Int Orthop* 2006 ; 30 : 478-483.
18. **Miles CA, Sionkowska A, Hulin SL *et al*.** Identification of an intermediate state in the helix-coil degradation of collagen by ultraviolet light. *J Biol Chem* 2000 ; 275 : 33014-33020.
19. **Mitchell EJ, Stawarz AM, Kayacan R, Rimnac CM.** The effect of gamma radiation sterilization on the fatigue crack propagation resistance of human cortical bone. *J Bone Joint Surg* 2004 ; 86-A : 2648-2657.
20. **Nemzek JA, Arnoczky SP, Swenson CL.** Retroviral transmission by the transplantation of connective-tissue allografts : an experimental study. *J Bone Joint Surg* 1994 ; 76-A : 1036-1041.
21. **Simonian PT, Conrad EU, Chapman JR *et al*.** Effect of sterilization and storage treatments on screw pullout strength in human allograft bone. *Clin Orthop Relat Res* 1994 ; 302 : 290-296.18.
22. **Spire B, Barre-Sinoussi F, Montagnier L, Chermann JC.** Inactivation of lymphadenopathy associated virus by chemical disinfectants. *Lancet* 1984 ; 20 : 899-901.
23. **Zhang Q, Cornu O, Delloye C.** Ethylene oxide does not extinguish the osteoinductive capacity of demineralized bone. A reappraisal in rats. *Acta Orthop Scand* 1997 ; 68 : 104-108.