

Effects of gamma irradiation on mechanical properties of defatted trabecular bone allografts assessed by speed-of-sound measurement

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Abstract New sterilization methods for human bone allografts may lead to alterations in bone mechanical properties, which strongly influence short- and medium-term outcomes. In many sterilization procedures, bone allografts are subjected to gamma irradiation, usually with 25 KGy, after treatment and packaging. We used speed-of-sound (SOS) measurements to evaluate the effects of gamma irradiation on bone. All bone specimens were subjected to the same microbial inactivation procedure. They were then separated into three groups, of which one was treated and not irradiated and two were exposed to 10 and 25 KGy of gamma radiation, respectively. SOS was measured using high- and low-frequency

ultrasound beams in each orthogonal direction. SOS and Young modulus were altered significantly in the three groups, compared to native untreated bone. Exposure to 10 or 25 KGy had no noticeable effect on the study variables. The impact of irradiation was small compared to the effects of physical or chemical defatting. Reducing the radiation dose used in everyday practice failed to improve graft mechanical properties in this study.

Keywords Defatted Bone · Mechanical properties · Gamma irradiation · Ultrasound wave propagation

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Introduction

The recent emergence of new communicable diseases, most notably HIV infection (Smith et al. 2001) and variant Creutzfeldt–Jakob disease, has focused attention on the procedures used to sterilize tissue allografts. Conventional techniques include irradiation, heating, and freeze-drying (Anderson et al. 1992; Cornu et al. 2000; Grieb et al. 2005; Loty et al. 1990). Sophisticated methods for sterilizing bone allografts have been developed over the last few years (Chappard et al. 1993; Kalus et al. 2005; Mitton et al. 2005; Mroz 2006; Poumarat and Squire 1993). They seek to achieve a high level of

safety by using several methods in sequence. The first step consists in defatting and dehydration, which virtually eliminates the bone marrow. Then, procedures for inactivating bacteria and viruses are applied. Protein denaturation may be used to inactivate prions. Finally, the graft is packaged and sterilized, usually by gamma irradiation (Carter and Hayes 1976; Kalus et al. 2005; Mitton et al. 2005; Mroz 2006; Poumarat and Squire 1993). Among these treatments, some lead to significant changes in bone properties. Thus, a 35–40% decrease in mechanical resistance has been reported (Vastel et al. 2004). However, the respective contributions of chemical treatments and final irradiation to the changes in bone properties remain unknown.

Little is known about the possible effects of gamma irradiation on the mechanical properties of dehydrated bone. Modest alterations in mechanical strength of fresh-frozen bone specimens occurred after gamma irradiation with 60 KGy, but not with lower doses (Anderson et al. 1992). Possible effects of gamma irradiation on defatted dehydrated bone have not been addressed specifically. In patients undergoing bone grafting, stability of the fixation remains dependent on the mechanical strength of the graft until osteointegration is sufficient to absorb the mechanical loads (Crowninshield et al. 1983; Oonishi et al. 1983). The objective of this study was to evaluate the effects of two gamma radiation doses, 10 KGy and 25 KGy, on the mechanical properties of defatted, dehydrated, treated bone with the goal of determining whether lower radiation doses were associated with better preservation of the mechanical properties of allogeneic bone grafts.

Material and methods

Bone specimen collection and preparation

The bone specimens were taken from femoral heads removed during multiple organ harvesting, in compliance with French regulations. The donors were nine men and five women whose ages ranged from 23 to 58 years. Between steps in the treatment process, the bone fragments were

immersed in saline and frozen at -40°C . A previously published method was used to prepare the specimens (Vastel et al. 2004). Briefly, the femoral heads were pared using an oscillating saw, along the main load-bearing axis during life. Then one or two 9 mm-thick slices including the centre of the femoral head were cut using a low-speed saw with continuous saline irrigation. Four parallelepipeds were cut from the central part of each slice, placed in their native position, and numbered (Fig. 1). Methylene blue marks were made on each specimen to show its position in the body relative to the direction of mechanical loads. The three dimensions of the specimens were measured using digital callipers. Sixty specimens were prepared for the study.

Speed-of-sound measurements

A Sofranel[®] (Pulser Receiver Model) device was used to generate ultrasound waves. Speed of sound (SOS) was determined using low-frequency sensors (Physical Acoustics Group[®]) and high-frequency sensors (Ultran[®], WS 75-2). After signal amplification, the time needed for sound-wave propagation was measured using a digital oscilloscope (TDS 210, Tektronics[®], Beaverton, OR, USA). The data were stored in a computer run using Microsoft Windows and Excel and equipped with a Tekvisa data acquisition interface.

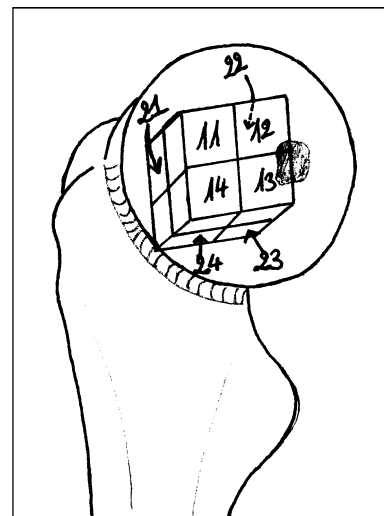


Fig. 1 Collection and numbering of the specimens from the centre of the femoral head

The time needed for sound-wave propagation was defined as the difference between t_1 and t_2 , where t_1 was related to the maximal value of the input signal delivered by the ultrasound generator, and t_2 was the time the signal reached the second probe. Signal noise was determined by performing the measurements without specimens. SOS at each of the two frequencies was measured along three mutually orthogonal planes, one of which was the plane of greatest mechanical loads during life.

The specimens were thawed at 4°C for 16 h before the measurements. The number of freeze-thaw cycles was the same for all specimens. For each measurement session, 40 specimens were thawed. All measurements were performed in the same room, by the same operator, at a constant temperature of about 20°C. Within-run reproducibility was determined using calibration standards and anatomic bone pieces. The standards were made of aluminium, stainless steel, copper, and brass. They were used at the beginning of each run to validate the measurement conditions and to check the performance of the measurement devices. Each of the anatomic bone pieces was tested six times along each of the three planes, at both frequencies. To determine the best method for hydrating the specimens, two procedures were compared: in one procedure, the specimen was immersed in saline then placed on the sensor, the mean time between removal from saline and measurement being 30 s; in the other procedure, the specimen was immersed in saline then dried on towel paper, and the mean time from saline removal to measurement was 1 min.

Processing methods

All the specimens were subjected to fat extraction by supercritical carbon dioxide followed by inactivation using sequential exposure to hydrogen peroxide, sodium hydroxide, monosodium dihydrogen phosphate, and ethanol (Mitton et al. 2005). The specimens were then divided into three groups. The control specimens received no further treatment. The other two groups were subjected to gamma irradiation in a dose of 10 KGy and 25 KGy, respectively. Each group was successively tested with low and high frequency sensors, which yielded six subgroups of results.

Density measurements(σ)

To evaluate the modulus of elasticity from low-frequency measurements, the apparent density of each specimen was determined. A precision balance (0.01 g) was used to measure the mass of each treated dehydrated specimen. The apparent total volume of each specimen was computed from the lengths of the three dimensions of the parallelepiped, which were measured using micrometric callipers.

Determination of the modulus of elasticity

The modulus of elasticity was computed using the equation:

$$E_i = \sigma v^2 i$$

where E_i is the modulus of elasticity, σ the apparent density, and v the velocity of the ultrasound waves (Abendstein and Hyatt 1970; Ashman et al. 1987).

Statistics

All statistical tests were run using R software (R Foundation for Statistical Computing, Vienna, Austria). The Shapiro–Wilk test for normality consistently showed heterogeneity across the three measurement planes, the three specimen groups, and the two sound-wave frequencies. We therefore used non-parametric tests. The Wilcoxon test was chosen for comparing two paired groups and the Friedman test for comparing more than two paired groups. Values of P smaller than 0.05 were considered significant. Correlations were evaluated using the Pearson test, with P values smaller than 0.05 being considered significant.

Results

Determination of the best procedure for specimen hydration

Variability was 0.96% (SD: 0.36%) without towel paper and 3.90% (SD: 2.07%) with towel paper.

Therefore, we used the simpler method without towel paper for our experiments.

Baseline SOS values in each of the three groups are reported in Table 1. The three groups were not significantly different ($P = 0.2105$, Friedman test). The mean values were also similar in the three groups. SOS values after treatment are shown in Table 2 and mean differences between baseline and post-irradiation values in Table 3.

Mean variations of modulus of elasticity values, calculated on both SOS and density measurements and compared in each group to values obtained using fresh bone, are reported in Table 4. Due to the limited precision of the

density measurements (2.1%) and SOS measurements (2.8%), the variations were not statistically significant.

Discussion

SOS correlates with the elasticity modulus (Abendstein and Hyatt 1970; Ashman et al. 1987) and with mechanical strength as assessed by failure stress determination (Abendstein and Hyatt 1970; Vastel et al. 2004). Thus, evaluations of SOS and of the elasticity modulus are useful as non-destructive means of obtaining information on mechanical strength.

Table 1 Speed of sound in the three groups of specimens before treatment

Mean (SD) m/s	LF Load plane	LF Ortho 1	LF Ortho 2	HF Load plane	HF Ortho 1	HF Ortho 2
No irradiation ($n = 20$)	2,343 ± 95	2,137 ± 164	2,189 ± 177	2,728 ± 82	2,472 ± 173	2,544 ± 189
10 KGys ($n = 20$)	2,342 ± 97	2,163 ± 122	2,194 ± 182	2,721 ± 108	2,505 ± 122	2,567 ± 214
25 KGys ($n = 20$)	2,343 ± 101	2,102 ± 127	2,152 ± 206	2,735 ± 103	2,451 ± 150	2,511 ± 244

HF—high frequency; LF—low frequency; Load plane—plane in which the main loading forces occurred during life; Ortho 1 and Ortho 2: planes orthogonal to the load plane and to each other

Table 2 Speed of sound through treated non-irradiated bone and through treated bone exposed to 10 KGy or 25 KGy of gamma radiation

V:m/s (SD) Δv (%)	LF Load plane	LF Ortho 1	LF Ortho 2	HF Load plane	HF Ortho 1	HF Ortho 2
Defatting alone	2,272 ± 114 (−3.03%)	2,054 ± 158 (−3.88%)	2,131 ± 164 (2.64%)	2,642 ± 84 (−3.15%)	2,400 ± 196 (−2.91%)	2,484 ± 186 (−2.36%)
Defatting + 10 KGy	2,274 ± 105 (−2.9%)	2,049 ± 167 (−5.64%)	2,100 ± 209 (−4.28%)	2,653 ± 93 (−2.45%)	2,386 ± 192 (−4.75%)	2,453 ± 245 (−4.44%)
Defatting + 25 KGy	2,281 ± 85 (−2.9%)	2,126 ± 114 (+1.12%)	2,140 ± 204 (−0.56%)	2,673 ± 111 (+1.17%)	2,475 ± 136 (+0.98%)	2,521 ± 218 (+0.4%)

HF—high frequency; LF—low frequency; Load plane: plane in which the main loading forces occurred during life; Ortho 1 and Ortho 2: planes orthogonal to the load plane and to each other

Table 3 Statistical significance of observed differences in speed of sound induced by exposure to 10 KGy or 25 KGy of gamma radiation

p value (Friedman test)	LF Load plane	LF Ortho 1	LF Ortho 2	HF Load plane	HF Ortho 1	HF Ortho 2
Control/10 KGy	0.1	1.3	0.3	0.2	0.3	0.1
Control/25 KGy	0.5	2.2*	0.1	1.4**	1.7*	0.5

HF—high frequency; LF—low frequency; Load plane: plane in which the main loading forces occurred during life; Ortho 1 and Ortho 2: planes orthogonal to the load plane and to each other

* $P < 0.05$, Wilcoxon test

** $P < 0.01$, Wilcoxon test

Table 4 Mean variation of modulus of elasticity after treatment compared to fresh bone

% <i>N</i> = 20	E Load plane	E Ortho 1	E Ortho 2
Defatting alone (SD) (min/max)	+ 5.9%(5.4) (-4.0/ + 20.8)	+ 7.5%(4.8) (-0.8/ + 19.2)	+ 5.1% (4.6) (-6.7/ + 14.1)
Defatting + 10 KGy (SD) (min/max)	+ 5.7%(4.4) (-2.9/ + 14.8)	+ 4.7%(10.2) (-20.9/ + 25.9)	+ 4.5% (8.9) (-24.0/ + 25.7)
Defatting. + 25 KGy (SD) (min/max)	+ 5.0%(4.6) (-2.3/ + 13.1)	+ 3.3%(5.4) (-9.8/ + 10.7)	+ 4.9%(6.3) (-9.3/ + 14.4)

A correlation between the modulus of elasticity and SOS was established for fresh bone (Abendstein and Hyatt 1970; Ashman et al. 1987), Carter and Hayes (1976) reported that the intertrabecular marrow had no effect on the mechanical properties of cancellous bone. Using conventional mechanical tests, Hodgkinson [Oonishi et al. 1983] found a tight correlation between SOS in fresh bone and the modulus of elasticity of defatted bone. Also using conventional mechanical tests, we previously noted a close correlation between SOS and breaking stress (Hodgkinson et al. 1997). These data indicate that our comparative results reflect alterations in mechanical properties induced by the study treatments.

Our results are consonant with those obtained in earlier studies (Ashman et al. 1987; Njeh et al. 1997). The slightly higher values in our study may be ascribable to the young age of our donors (mean, 46 years), since the mechanical strength of trabecular bone decreases with age (Smith and Smith 1976; Weaver and Chalmers 1966). The within-run reproducibility tests allowed us to select the optimal measurement conditions and to check the reliability of our measurements. We compared SOS values at baseline and after processing in order to eliminate confounding due to bone density variations across individuals [Weaver and Chalmers 1996] or across bone sites in a given individual (Brown and Ferguson 1980). Thus, our non-destructive method produced direct evaluations of the impact of processing on trabecular bone. As previously reported (Deligianni et al. 1994; Njeh et al. 1997), we found differences between the plane aligned with mechanical loads during life and the two other planes. These results indicating anisotropic behaviour of the bone specimens validate the collection, preservation, and preparation procedures used in our study.

In our study, exposure of treated dehydrated bone specimens to 10 KGy or 25 KGy of gamma radiation did not cause major alterations in mechanical properties. SOS changes were about 2% of the values in non-irradiated bone; in no instances did they exceed 3.5%. The major discrepancies between SOS values and values obtained using conventional mechanical tests should be borne in mind. We previously found changes in breaking stress of about 35% coinciding with an only 6.2% decrease in SOS, with 2.5% differences in SOS corresponding to an about 10% change in breaking stress (Vastel et al. 2004). These findings indicate that gamma irradiation induces limited but generally measurable changes in the allograft. Our protocol was identical to that used in a previous study (Vastel et al. 2004) showing an about 11% change in breaking stress when SOS changed by 2–3%. This point is important, as allografts must be fashioned before implantation in order to obtain a stable fixation. Surgeons often complain that cleansed dehydrated allografts are brittle. However, most of the changes induced by the entire treatment process seem ascribable to the defatting steps, with subsequent radiation exposure having a minimal impact. Furthermore, lower radiation doses may be associated with decreased sterilizing efficacy. Consequently, our data support irradiation with 25 KGy, which is the dose currently used in most industrial preparation processes.

Conclusion

Our study of the speed of high- and low-frequency sound waves travelling through bone found that gamma irradiation with 10 KGy or 25 KGy induced moderate modifications in mechanical properties, compared to treated

non-irradiated bone. However, exposure to 10 or 25 KGy was followed by very small decreases in Young's modulus and SOS in some of the specimens, barely larger than the margin of error of our measurements. Decreased allograft resistance may complicate surgery, and consequently studies are needed to improve the mechanical resistance of treated allogeneic bone grafts. Our results indicate that reducing the radiation dose from 25 KGy to 10 KGy is not effective in improving the mechanical quality of allogeneic bone grafts.

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