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Effect of different sterilization processing methods on the mechanical properties of human cancellous bone allografts

L. Vastel[a,*], A. Meunier[b], H. Siney[c], L. Sedel[b], J.-P. Courpied[a]

[a] *Bone Tissue Banking, Cochin AP-HP, Service de Chirurgie Orthopédique A, Hôpital Cochin, AP-HP, 28 rue du Faubourg Saint Jacques, 75014, Paris Cedex 14, France*
[b] *Laboratory of Orthopedic Research/UPRES-A CNRS 7052, Faculty of Medicine Lariboisière, Paris, France*
[c] *Biostatistics Department, Faculty of Medicine, Cochin, Paris, France*

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Abstract

Use of new sterilization methods applied to human bone is likely to affect both the mechanical and biological properties of human cancellous grafts. The mechanical properties of the transplanted bone inevitably determine the short- and mid-term results of the orthopedic procedure performed. The aim of this study was to compare, under similar conditions, the mechanical effects of gamma irradiation, lipid extraction, and treatment with 6 M urea on trabecular bone samples, through conventional mechanical tests and measurement of the ultrasound wave propagation rate. Deteriorations measured for gamma irradiation and lipid extraction were low: 2.4% and 2.5%, respectively, for ultrasound propagation wave measurements. They were clearly significant for protocol including 6 M urea, corresponding to a loss of 30% in values measured in the control sample for the stress to failure, inciting prudence when grafted bone is used for support in orthopedic assembly. High consistency in the results obtained between travel time of the ultrasound wave, easily done, and measurement of stress to failure through conventional tests, favor the use of ultrasound protocol, described as a quality test performed on bone grafts in the tissue bank before distribution and implantation.

Keywords: Trabecular bone; Allografts; Sterilization methods; Mechanical properties

1. Introduction

The use of human cancellous bone grafts is now routine practice in orthopedic surgery, especially in hip revision and tumor or trauma surgery. Transplanted bone is obtained, in almost all cases from patients in whom the femoral head was collected during total hip arthroplasty, and is implanted after a safety of use assessment, performed within a tissue bank.

As a result of the occurrence of new transmissible diseases (bovine spongiform encephalopathy, hepatitis, etc.), concerns regarding safety of bone grafts have increased enormously over the last few years, in particular in cases of non-vital procedures often involving young patients.

The need to maintain maximum safety has resulted in the use of new sterilization methods [1–4] applied to human bone, and in more systematic use of some methods previously known for several years [5–7]. Currently, the most commonly used methods are irradiation procedures (beta and gamma) [8], for bactericidal and virucidal purposes, now used in some processing methods with chemical agents (NaOH or 6 M urea), with a view to denaturing unconventional transmissible protein agents (PRION).

These processing methods may follow lipid extraction (cleaning of non-mineralized tissue and excision of the bone marrow), thus enabling optimization of these procedures; lipid extraction according to some authors [9] is reputed to promote the process of subsequent osteointegration of the bone graft. Such processing methods are likely to affect both the mechanical and biological [2] properties of the trabecular bone.

However, in some cases, the mechanical properties of the transplanted bone graft inevitably determine the

*Corresponding author. Tel.: +33-158-413-037; fax: +33-158-413-115.
E-mail address: laurent.vastel@cch.ap-hop-paris.fr (L. Vastel).

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short- and mid-term results of the procedure performed. Thus, the aim of this study was to compare the effect of different sterilization processing methods, currently used in routine practice, on the mechanical properties of trabecular bone through conventional mechanical tests and measurement of the ultrasound wave propagation rate. The treatments studied were: gamma irradiation, mechanical lipid extraction, and treatment with 6 M urea.

2. Material and methods

2.1. Source of the test bone

Eighteen femoral heads were collected from 14 cadavers of 19–60 years of age, free from any known disease of the hip, in the context of multiorgan collection. Femoral heads were frozen immediately after excision. They were packaged without any additives in sterile, airtight, triple water-resistant plastic package and stored at −80°C until use.

2.2. Preparation of test samples

Considering interindividual variations in mechanical properties, matched comparisons only concerned samples from a same femoral head [10,11].

Taking into account the mechanical property distributions in the cancellous bone of the femoral head [12,13], matched samples were prepared from a restricted central area of the head, in which homogeneous mechanical properties could be supposed in native bone (Fig. 1). In this area, and in each femoral head, four cubes of identical volume were prepared, 9 mm thick. Ancillary equipment enabled obtention of strictly parallel 2×2 cutting planes. Seventy-two samples were obtained consisting in 18 groups of 4 samples.

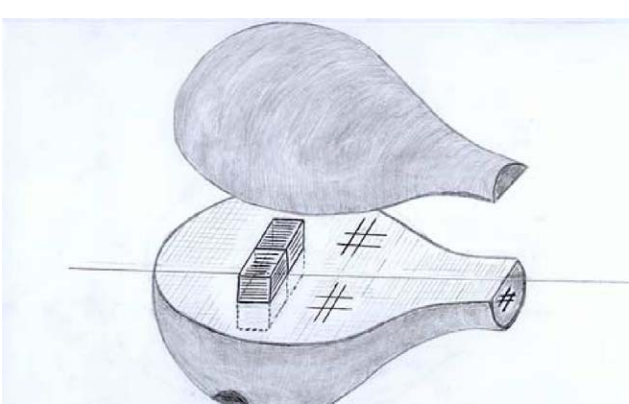

Fig. 1. Samples preparation, cut from a restricted central area of the femoral head.

2.3. Processing methods applied

In each group of 4 samples from a given femoral head, the samples underwent one of the following processing methods:

The control sample did not undergo any additional processing and was returned to the freezer at –80°C, in a test tube with physiological saline.

Lipid extraction: The samples involved were cleaned by pressure irrigation with physiological saline (90 bars) and were then immersed in a solution of pure acetone (50 ml) for 17 h in two successive baths. Then, the samples were returned in a test tube of physiological saline to the freezer.

Processing with 6 M *urea*: These samples underwent the different steps of lipid extraction as described above. They were then rinsed with pure water and immersed successively in a detergent solution and in 6 M urea solutions for a total of 2×3 h. After rinsing with pure water they were finally dried under a hot (37°C) air stream for 12 h. Before the first tests they were rehydrated in physiological saline during 3 h, and after the tests they were kept in physiological saline at −80°C.

γ-Irradiation: The samples involved were defrosted in the test tube and then irradiated with a dose of 30 kGy (from 29.3 to 36.3). They were then refrozen at −80°C.

2.4. A circular permutation between samples and treatments was undertaken

γ-Irradiation was attributed to sample A in the first group, B in the second, C in the third, etc.

In each group of 4 samples, the 4 treatments coexisted, and comparisons were realized between treated samples and the control sample of the group.

2.5. Measurement of the ultrasound wave propagation rate

The conduction rate was calculated, based on measurement of the ultrasound wave travel time within each sample. Each sample was directed to its reference anatomical orientation, in order to undergo testing aligned to the axis of transmission of body load stress in the living subject. Its thickness was measured using a micrometer (accuracy: 10 μm). A Sofranel® apparatus, model 5052 UA, operating in transmission mode, with a distinct transmitter and receiver, at a 2.25-MHz frequency, generated ultrasounds.

Measurement of transmission time was obtained after parameterizing the apparatus with a 5-mm thick Plexiglas puck.

These measurements were obtained after gradual and complete defreezing of the sample (3 h at room temperature). The sample was then kept moist during the different handling procedures. Measurement of the transmission time in the different samples was carried out before and after processing.

Measurements before processing allowed us to evaluate, by a non-destructive procedure, the potential variations between test cubes from a given group of 4 samples from a same femoral head, and to validate the model selected.

The measurements obtained after processing allowed us to perform, for each sample, a matched comparison of conduction rate before and after processing.

The samples were then returned to the test tubes filled with physiological saline and refrozen at -80°C.

2.6. Mechanical tests

The machine used was a screw-filled tensile compression machine (Wolpert®). Each sample was deposited on the plate of the machine according to its anatomical orientation. Compression was applied at a rate of 2 mm per minute, with the mobile plate beginning its travel at a short distance from the sample but having acquired its rate when contact was made with the upper aspect of the sample. The machine sensors allowed measurement of the travel and compression stress throughout the procedure. An A/D card connected to a microcomputer was used to store data. Acquisition (8 points/s) was carried out using a special software specific to the card (Winview®). Data were then transferred to a spreadsheet (Excel, Microsoft®). For each sample, the test was interrupted once the first deflection of the stress/strain curve was obtained.

Deformation at the time of failure was measured, and elasticity modulus was calculated in the first, straight section of the strain deformation curve.

2.7. Statistical analysis

Ultrasound wave conduction rate: A comparison was performed on the conduction rate in the groups before any treatment.

After processing we compared the conduction rate before and after processing for each sample. The difference determined the delta-VC, which was the parameter studied for statistical tests.

The parameters studied were stress to failure, deformation at the time of failure, and elasticity modulus.

A non-parametric analysis of variance was performed (two factors: Groups of 4 samples, treatment) for each factor studied (Friedmann's test). When the test was positive, match comparisons were realized between each treatment studied and Newmann–Keuls test was used.

The α risk was defined at 5% level.

3. Results

3.1. Data concerning the delipidation group was lost as a consequence of a handling mistake

3.2. Results of measurements performed:

The mean results ($\pm$SD) are resumed in Table 1.

Before treatment, conduction rate at the time of inclusion was not significantly different between the four groups ($p = 0.32$). After treatment analysis of variance was significant for each parameter.

For the Delta VC parameter, the Newmann–Keuls test found a significant difference between the control group and the treated groups (Fig. 2). The results of the 6 M urea treated group were significantly different from the other results obtained with the other treatment and from the control group. No significant difference was

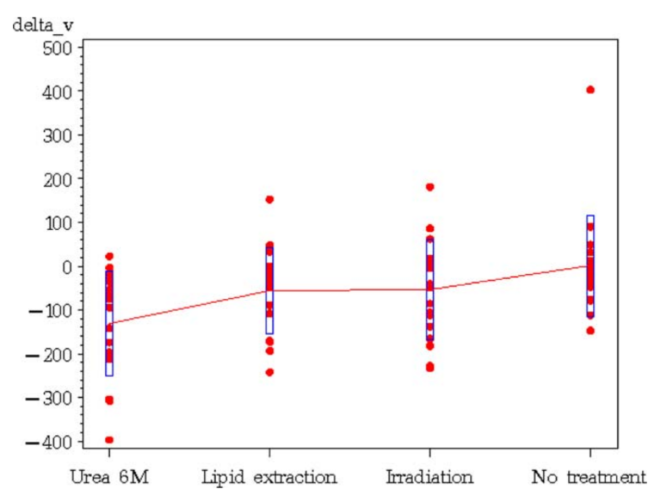

Fig. 2. Delta V before and after treatments.

Table 1
Mean results of the tests performed

Group	No treat.	Urea 6 M	Lipid extr.	Irradiation	p
V before TT ($\pm$SD)	2245$\pm$150	2232$\pm$152	2264$\pm$147	2229$\pm$150	0.32
V after TT ($\pm$SD)	2246$\pm$115	2100$\pm$173	2209$\pm$143	2175$\pm$125	
Delta VC ($\pm$SD)	0.4$\pm$115.4	$-$131.6$\pm$118.8	$-$55.7$\pm$98.5	$-$53.6$\pm$113.4	$<10^{-4}$
Sigma failure ($\pm$SD)	13.5$\pm$3.1	8.9$\pm$3.9	12.2$\pm$4.1	12.3$\pm$4.3	4.3×10^{-3}
Eps failure ($\pm$SD)	3.1$\pm$1.0	2.5$\pm$0.6	3.2$\pm$0.8	2.7$\pm$0.9	$<10^{-4}$
E ($\pm$SD)	876.8$\pm$331.6	646.1$\pm$359.0	761.9$\pm$268.0	817.2$\pm$282.5	3.3×10^{-2}

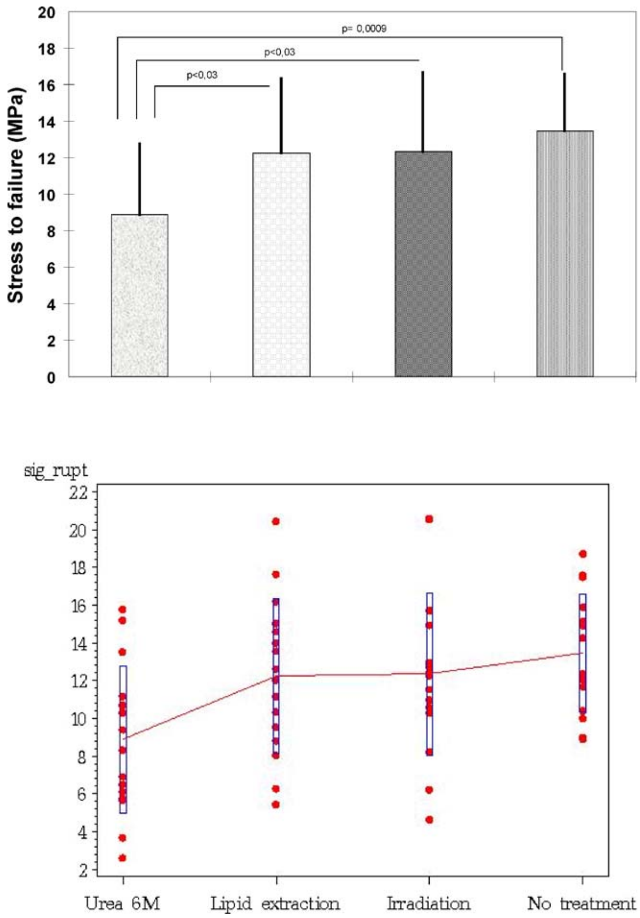

Fig. 3. Mean results of the stress to failure in the different treated groups.

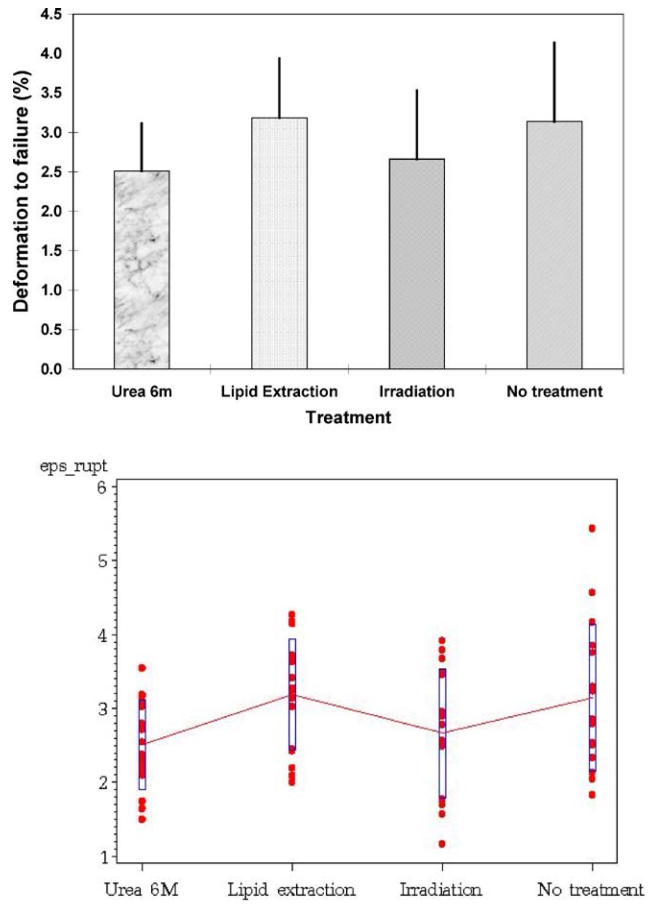

Fig. 4. Mean results of the deformation to failure in the different treated groups.

found between the group treated by lipid extraction and the irradiated group, but results were significantly different in one part from those obtained with the 6 M urea treated group, and from those obtained with the control group in a second part. For the measurement of deformation at the time of failure and stress to failure (Figs. 3 and 4), 6 M urea was significantly different from the others. No significant difference was observed between irradiation, lipid extraction and the control group.

4. Discussion

4.1. Measurements of rate of travel of the ultrasound wave

Abendstein et al. [14] found a constant relationship between the modulus of elasticity during compression determined by ultrasounds and the modulus of elasticity measured mechanically. Ideally, this formula can only be applied if the acoustic length is clearly greater than the mean diameter of pores and the dimensions of the surface area of a specimen value [15,16]. The value of the stress to failure is also closely related to the propagation rate measured [14]. Samples tested by these methods should be of adequate length. Ashman [15] proposed cubes of 12 mm on each side and advised against the use of samples not allowing a propagation of at least 5 mm. The transmission frequency used in our study, i.e., 2.25 MHz, corresponds to a 1-mm wavelength, greater than the mean diameter of pores in human trabecular bone (0.1–0.5 mm). This value is similar to those used in other published series, but it cannot claim to attain Young's modulus, considering the dimensions of the sample that are greater than the acoustic wave length.

4.2. Test conditions

Samples were tested after complete gradual thawing at room temperature. They remained moist throughout all handling procedures. Conduct of all tests in a continuous session before processing and then in a continuous session after processing, for ultrasound wave and for mechanical tests, allowed us to assume that conditions for measurement remained constant. The lack of difference, regarding the ultrasound wave travel rates within the samples collected from a given femoral head, seemed to confirm that the choices made for preparation of the samples were valid. The choice of samples obtained from the central part of each femoral head (oriented in accordance with their situation in vivo), of small size, allowed the obtention of samples with identical characteristics, which would not be possible with samples of greater size. Thus mechanical test comparisons could be conducted, with the control sample, for each group of samples and liberation from inter-subject differences could be ensured [10,12,17].

4.3. Mechanical tests

Because of the speed used in this study, which was less than 10 m/s, the effect of the fluid phase, on the results obtained, need not be taken in account [18,19]. Stress direction on the studied samples, being orientated in accordance with its initial physiological situation, was consequently identical for all the samples tested [12,20,21]. Even though care was taken to grease the machine plates, the stresses of friction on the interface cannot be neglected—a reminder of the limits of any mechanical compression test. However, the results obtained were of the same order of magnitude as those reported in the literature [13,18,20], and the aim of the study was not to characterize trabecular bone as such, but rather to evaluate the effect of different sterilization processing methods on the mechanical properties of processed trabecular bone tested under similar conditions.

The protocol of rehydration and conservation between tests in physiological saline is questionable, but constitutes the usual method applied in surgery, and the protocol was identical for all the treatments tested, particularly for delipidation and delipidation together with 6 M urea treatment. The significant differences in the results of mechanical tests, observed between these two methods, cannot be attributed to the rehydration protocol.

4.4. Discussion of the results

A high consistency in the results obtained was observed between the measurement of travel time of the ultrasound wave and those carried out in the setting of mechanical tests: stress to failure and deformation to failure. This consistency of results is in agreement with the data in the literature [15,18,19].

Gamma irradiation at the doses used only slightly altered the measurements performed on the bone, which underwent processing. This result is consistent with the rare data in the literature [4,8]: the latter do not mention any significant effect of gamma irradiation at the doses used in orthopedic practice. In our context, the deterioration in the ultrasound wave conduction rate was poor (2.4%), but significant. The results obtained tends to demonstrate an effect of irradiation on trabecular bone tissue, which is also detected by conventional mechanical tests and which was not previously reported.

Lipid extraction produced a significant decrease in the acoustic conduction rate (2.5%); this variation was low and was also measured by mechanical tests.

Processing with 6 M urea significantly deteriorated the measurements performed on the bone which underwent processing; the fall in the travel rate was nearly 6% and the mean stress to failure showed important (34%) and significant differences between the different groups.

Comparative results obtained with the two methods used are consistent with a low structural deterioration when using irradiation, slightly lower (and significant) for lipid extraction, and high when processing with 6 M urea. These hypotheses are compatible with the action mechanism of the different processing methods used: physical processing for irradiation, which, at the doses used, does not greatly alter the protein structure of the material exposed. Lipid extraction, thus weakly activates denaturing agents. Urea acts by deteriorating the tertiary structure of proteins. At a 2 M concentration, it is already able to produce a major deterioration in globular proteins [22]. Fibrillar proteins, such as type I collagen, which is more resistant to the denaturing process, can be deteriorated at concentrations as high as those used (6 M). This is in agreement with the amplification observed for variations in the value of the stress to failure, suggesting a processing-related architectural deterioration, in relation to denaturing of the collagen framework, which is not observed with conventional treatments [4,8,23].

A high level of safety, including the process against "unconventional" protein agents such as the prion, necessarily alters the framework structure of treated bone and reduces its mechanical properties.

At present, the trabecular allograft is most frequently used in orthopedic surgery for acetabular reconstruction in reinforcement of prosthetic total hip replacement. The mean stresses tolerated by a hip are approximately 1–3 MPa in the acetabulum [12,18,24,25], with peak stresses which can reach up to 8.8 MPa [12]. The success of reconstruction depends on the resistance of the material employed to complete graft osteointegration: this must occur within a sufficiently rapid time period, i.e., less than the limits of resistance to fatigue of the reinforcement material used. From a mechanical standpoint, use of the reinforcement acetabular device seems to be very important, particularly when treated bone is used for the bone reconstruction.

From the standpoint of the tissue bank, such processes will not be applied to produce solid pieces when grafts are provided by old donors with osteoporosis [26], for example in the case of fracture of the femoral neck.

5. Conclusion

Various deteriorations associated with processing were following investigation of the mechanical properties of trabecular bone from human femoral heads and evaluation of the effect of different sterilization processing methods. Measurement of the ultrasound wave propagation rate in the samples revealed a deterioration of 2.4% in the rate measured, compared to controls, resulting from gamma irradiation at doses of 30 kGy, which was significant. It revealed deterioration of 2.5% in the rate measured, which was significant, for lipid extraction with acetone and alcohol. It revealed 5.9% deterioration in the rate measured for complete processing with urea 6 M, which was highly significant. These deteriorations resulted in the same effects when measurement of the stress to failure was performed with conventional mechanical compression tests. Although these effects were significant, but low, for irradiation and lipid extraction in the samples observed, they were however clearly significant for urea 6 M, corresponding to a loss of 30% in values measured in the control sample for the stress to failure. In orthopedic surgery, care must be taken when using some treated bone graft with protocol including Urea 6 M, with systematic use of reinforcement device or osteosynthesis. High consistency in the results obtained between the measurement of travel time of the ultrasound wave and measurement of stress to failure incite us to use protocol with ultrasound as a routine quality test which can be performed before furnishing bone graft to the surgeon. Maybe this test will allow us to extend indications of bone procurement in multi-organ collection, particularly in older donors.

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