Freeze-dried irradiated bone brittleness improves compactness in an impaction bone grafting model

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Background Defatted bone chips with or without freeze-drying and irradiation have mechanical advantages as compared to fresh-frozen controls in in vitro models of impaction. These improved results have been ascribed to replacement of viscous bone marrow by saline and embrittlement of the freeze-dried bone by irradiation.

Material and methods To determine which of these hypotheses is correct, we compared the development of stiffness and compactness of morselized bone graft that had been: 1) fat-reduced with saline, and 2) fresh-frozen, solvent-detergent defatted, 3) freeze-dried irradiated and 4) not irradiated. We used 12 osteoarthrotic femoral heads to prepare these four batches of morselized bone, and impacted 18 samples from each batch in a cylinder. The frozen bone grafts were tested after thawing at room temperature for 2 hours and the freeze-dried grafts were tested after 30 minutes of rehydration. We monitored the development of compactness and stiffness of the material during impaction.

Results The stiffness of the freeze-dried irradiated bone was greater than that of the other three series after 10, 50 and 150 impactions. The freeze-dried bone chips that were not irradiated and the chips defatted with saline alone were less stiff than the fresh-frozen control after 150 impactions.

Interpretation The brittleness of freeze-dried irradiated bone, caused by loss of the capacity to absorb energy in a plastic way, increases the compactness and stiffness of the morselized grafts. Washing bone with saline alone or treating bone with solvent-detergent but no irradiation had no similar mechanical advantage and the bone did not impact better than fresh-frozen undefatted bone in our model.

In a previous study, we showed that freeze-dried irradiated cancellous bone was able to get impacted faster than its fresh-frozen controls (Cornu et al. 2003b). Two hypotheses were advanced to support this observation. First, the replacement of viscous bone marrow by saline in the freeze-dried bone might accelerate the compaction of the grafts (Höstner et al. 2001). Secondly, the embrittlement due to bone processing (Cornu et al. 2000) could be of value in impaction bone grafting.

In the present study, we evaluated the mechanical properties of impaction procedures using none fat-reduced bone with saline and solvent-detergent defatted, bone chips which had also been freeze-dried and irradiated.

Material and methods

Preparation of morselized graft

We procured 12 osteoarthrotic femoral heads from patients who had undergone a primary hip arthroplasty (Table 1). The cancellous bone was isolated by shaving the soft tissues (articular cartilage remnants and synovium), the cortical head and neck with a band saw. The heads were cut into four equal parts: anteromedial, anterolateral, posteromedial and posterolateral. Each part of the femoral head was randomized to one of four groups: fresh-frozen bone containing its original bone marrow, fat-reduced bone with saline, solvent-detergent defatted freeze-dried bone, and solvent-detergent freeze-dried irradiated bone. Each series was composed at random of the same number of head quarters from each location, considering each

Table 1. Origin of the material and weight loss on preparation of the morsellized grafts

Donor			Whole head		Freeze-dried irradiated		Freeze-dried nonirradiated		Fresh-frozen washed with saline		Fresh-frozen	
Name	Sex	Age	Weight (g)	Weight without cartilage (g)	Head quarter	Weight (g)	Head quarter	Weight (g)	Head quarter	Weight (g)	Head	Weight (g)
BN	F	80	62.0	32.4	AL	7.3	AM	8.1	PL	6.9	PM	8.1
BA	F	69	79.1	39.9	AL	10.2	AM	9.2	PL	9.4	PM	8.6
DM	F	58	73.4	35.8	PL	8.4	PM	8.4	AL	9.2	AM	7.8
DJM	M	56	65.3	31.1	AL	6.7	AM	7.6	PL	8.0	PM	7.1
FM	M	62	99.6	59.3	PL	12.4	PM	17.3	AL	12.5	AM	13.9
JL	F	81	57.1	31.6	PL	6.9	PM	7.1	AL	7.0	AM	11.1
KH	F	72	69.3	24.8	AM	6.1	PL	6.4	PM	4.7	AL	6.1
LD	F	73	78.1	42.3	AM	10.0	PL	10.4	PM	9.4	AL	10.3
MA	M	75	103.0	52.1	AM	11.6	PL	11.6	PM	13.5	AL	12.6
NE	M	54	110.1	55.5	PM	12.9	AL	13.0	AM	13.0	PL	13.8
PF	F	66	97.4	54.0	PM	12.9	AL	12.4	AM	12.9	PL	12.9
TB	F	66	97.8	45.8	PM	11.1	AL	11.0	AM	12.1	PL	9.2
Mean		67.5	82.7	42.1		9.7		10.2		9.9		10.1

donor and its gender. These quarters from each group were then morselized once, when wet without any defatting step, with the small rasps of the Noviomagus bone mill (Spierings, Nijmegen, NL). The size of the chips was mean 3.3 (SD 1) mm. Morselized grafts were then mixed and weighed to form 18 homogeneous samples. The fresh-frozen group containing its original marrow fat was regarded as the control group and stored frozen at –80 °C. The second group of morselized grafts was treated with warm saline (37 °C) twice: these were immersed for 3 min in 750 mL. Thereafter, they were gently dried with a surgical compress and stored frozen at –80 °C.

The last two groups were thoroughly washed under a jet of deionized water to remove bone marrow and blood cells. Lipid extraction and protein denaturation (prion inactivating procedure) were done by treatment with solvent-detergents (Delloye et al. 1987). Morselized grafts from both of these groups were also freeze-dried for 72 h (temperature of the condensor –80 °C, temperature of the chamber –30 °C, working vacuum 1×10^{-4} mm of Hg). The residual moisture of the bone was calculated by gravimetry to be 2.5% of the dry material (Sartorius MA 30, Goettingen, Germany).

Table 2. Weight lost in preparing the morselized grafts

Batch	Total weight (g)	Weight after treatment (g)	Reduction factor	Weight of each sample (g)
Fresh-frozen	121.5	121.5	1	5.0
Fresh-frozen, washed with saline	116.9	88.8	0.76	3.8
Freeze-dried, not irradiated	120.7	44.1	0.36	1.8
Freeze-dried, irradiated	115.0	41.5	0.36	1.8

Graft samples were immediately packaged under vacuum and preserved at room temperature. One of these two groups was not irradiated, while the other one was irradiated at a minimal dose of 25 kGy (maximum 30 kGy, cobalt 60 source).

In the three processed groups, the graft lost fat, marrow and water. The weight of 5 g fresh frozen graft was reduced to 3.8 g after fat reduction with saline and 1.8 g at the end of the freeze-drying process (Table 2).

Mechanical testing

The experimental protocol is based on a method described elsewhere (Bavadekar et al. 2001). Samples of morselized grafts were put into an aluminum tube with micro-holes to permit the draining of saline and fat during compaction. The impaction itself was produced by a mass of 455 g falling from a height of 1 m on a solid cylinder telescoping freely into the tube over the grafts.

The fresh-frozen morselized grafts (control and fat-reduced with saline groups) were thawed at room temperature, 2 hours before the mechanical test. The freeze-dried samples were rehydrated separately with saline (in their plastic vials) for 30 minutes before being tested. Each sample was loaded into the cylinder and tested. The impaction procedure was interrupted regularly (at 1, 3, 5, 10, 20 and every 10 impactions up to 150) to measure the height of the column of morselized grafts with a digital caliper and its stiffness. The compressive stiffness (or Emod, MPa) of the impacted grafts was measured by gentle compression of the impacted material at a speed of 0.5 mm/min in a testing machine (Zwick model Z50/TH3A, Zwick GmbH, Ulm, Germany). The load was measured by a 2 kN load cell and the displacement by an extensometer (Multisens, Zwick) placed across the tube and the impactor. These tests were limited to a range of 80 N of force (0.5 MPa) or 0.3 mm of displacement to avoid excessive compression. The cylinder of grafts was immediately unloaded after reaching one of these limits. Stiffness was calculated as the slope of the linear part of the curve (between 60% and 98% of the maximal load). The height and stiffness measurements were standardized and performed during one minute between impactions to avoid interference with the time-dependent mechanical properties of morselized grafts (Ullmark and Nilsson 1999). As the samples were used to determine the density, 18 measurements were made for up to 3 impactions, 14 for up to 10 impactions, 10 for up to 50 impactions and 6 for up to 150 impactions for each type of graft.

Density measurements

The apparent density of the impacted material was measured, using a pQCT (peripheral quantitative computed tomography machine, model XCT Research SA+, Stratec, Pforzheim, Germany). Densitometric changes in each series were determine in 4 samples impacted until the 3rd impaction, 4 until the 10th impaction, 4 until the 50th impaction and 6 until the final 150th impaction. The density value is expressed in g/cm^3.

Statistics

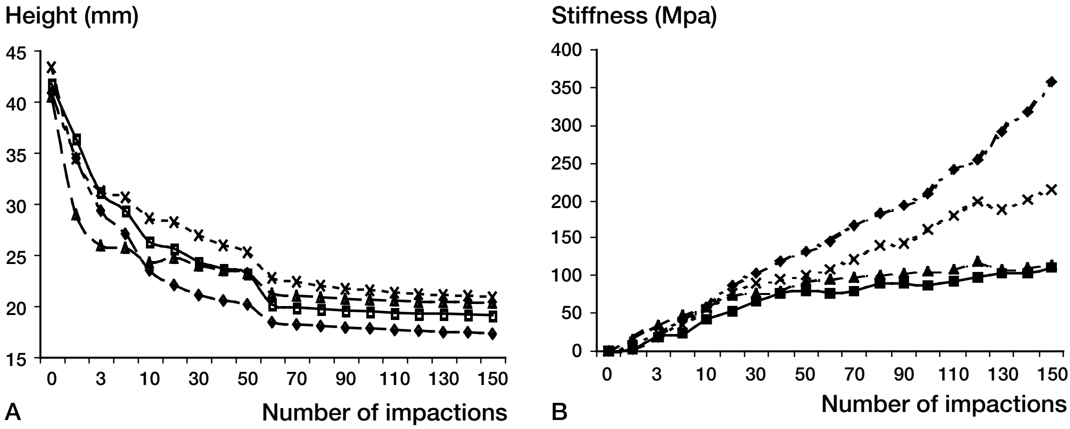
We used analysis of variance (ANOVA) with repeated measures (SPSS 10.0, SPSS Inc., Chicago, IL) to compare the results with the various types of grafts. The dependent variables were height and stiffness. The first within subject variable was type of grafts (freeze-dried or fresh-frozen). The number of impactions was introduced as a second within subject factor. Such model estimates of the overall behavior (the whole curve) differ when using one type of graft or another. This analysis was done considering the results from 1 to 3, 1 to 10, 1 to 50 and 1 to 150 impactions to assess the initial part of the impaction procedure. The two sample t-test was used to compare the estimates of density of both types of grafts.

Results

During impaction, the graft layers deformed quickly from 1 to 10 impactions and reached a steady state after 50 impactions in the four series. Grafts that had been defatted with saline and solvent-detergent treated grafts deformed more quickly than the fresh-frozen control grafts until 50 impactions ($p < 0.001$). From 50 up to 150 impactions, the solvent-detergent, defatted, freeze-dried, and irradiated graft became more compact than the other three groups ($p < 0.001$) (Figure A).

Stiffness tended to reach the maximum value after 120 impactions in the controls, the simply defatted with saline and the freeze-dried non irradiated groups. Stiffness of the freeze-dried irradiated group steadily increased during the entire impaction procedure (Figure B). Stiffness of the latter grafts was significantly greater than that of the other three series from 1 to 10, 1 to 50 and 1 to 150 impactions ($p < 0.001$; $p < 0.005$ as compared to the 1 to 150 impactions fresh-frozen control group). The stiffness of the fresh-frozen control grafts was greater than that of the simply defatted and the freeze-dried, but not irradiated groups after 150 impactions ($p < 0.001$).

The density increased during impactions in all the groups (Figure C). The mean density rose from a value of 0.34 g/cm^3 at 3 impactions to 0.59 g/cm^3 at 150 impactions in the three series that had not been irradiated. On the other hand, the



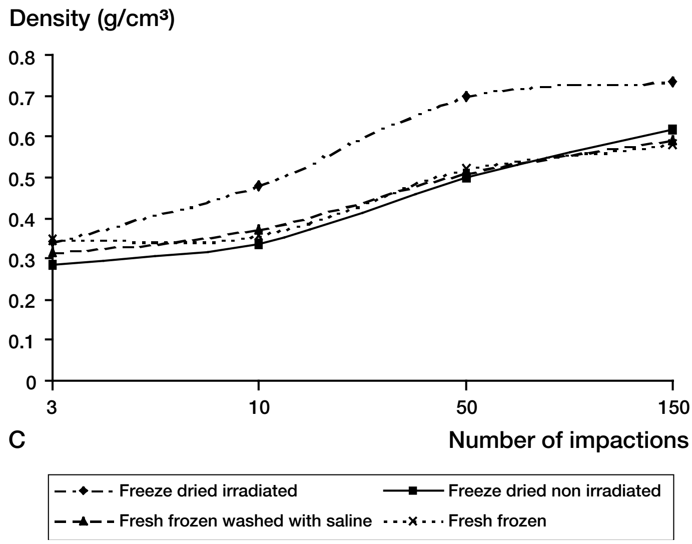


Figure. Defatted grafts compacted much faster than the fresh-frozen bone, as shown by the greater reduction in height from 1 to 50 impactions. After 10 impactions, freeze-dried irradiated bone compacted more than the other three series. The freeze-dried irradiated grafts were stiffer than the other three series from 10 to 150 impactions. The fresh-frozen control grafts were stiffer than those that had not been defatted and the freeze-dried groups that had not been irradiated after 150 impactions. The changes in density after 10 impactions showed that the reduction in height in the defatted series is due to bone embrittlement of the freeze-dried irradiated bone and to the loss of liquid from the defatted series that had not been irradiated.

freeze-dried irradiated morselized grafts showed a rapid increase in density from 0.34 g/cm^3 at 3 impactions to the final value of 0.73 g/cm^3 at 150 impactions. At 10, 50 and 150 impactions, the density was significantly greater in the freeze-dried irradiated bone ($p < 0.001$).

Discussion

In our previous studies, we have shown that femoral impaction reconstructions performed with freeze-dried irradiated bone are more stable than those performed with fresh-frozen bone in an in-vitro cyclic loading of cadaver femurs (Cornu et al. 2003a) and that freeze-dried irradiated bone simply became impacted much faster than the fresh-frozen controls in the same experimental model as those described in this paper (Cornu et al. 2003b). The first step of freeze-dried bone process is the removal of bone marrow and cell remains by solvent-detergent (Delloye et al. 1987). Many authors have reported an improvement in implant stability in femoral and acetabular impactions using a morselized graft that had been simply washed with saline (Kärrholm et al. 1999, Ullmark 2000, Höstner et al. 2001). In clinical studies, good results have also been reported using defatted freeze-dried bone (Mazhar et al. 2001, de Roeck and Drabu 2001, Thien et al. 2001). Replacing bone marrow by saline improves liquid flow, particularly after high speed stress (Carter and Hayes

1977), which may be helpful in impaction bone grafting. Our results did not support this finding, since grafts that had been washed with saline alone and solvent-detergent treated, freeze-dried, but not irradiated grafts did not impact better than fresh-frozen undefatted bone in our model, but defatted freeze-dried irradiated bone did. Marrow or liquid flow through the small holes in the aluminum tube may not be as complete and easily performed as in a bleeding diaphysis or other experimental models. Nevertheless, we observed a faster reduction in height in the three defatted series that may account for a rapid expulsion of liquid, but bone density and stiffness increased faster and to a higher value in the irradiated group. Moreover, in clinical work, graft impaction is rarely done without diaphyseal bleeding through the impacted material, which will reduce the benefits of washing in this aspect.

Gamma irradiation of solvent-detergent treated freeze-dried bone makes the material more brittle (Cornu et al. 2000). Since the stiffness of the graft layer depends on the compactness induced by the impaction (Bavadekar et al. 2001), an increase in brittleness will accelerate compaction. We have also seen that static compression of defatted freeze-dried bone reduces the ultimate strength and Young's modulus slightly, but does not affect work to failure, due to a higher plastic ductility (Cornu et al 2000). This higher ductility reduces the brittleness and may account for the lower compaction, stiffness and density of the freeze-dried bone that is not irradiated. Such a view accords with a comparison of the results of this study to the previous one on freeze-dried irradiated bone using the same model (Cornu et al. 2003b). The decrease in the height of the layered grafts and the increase in the density and stiffness occurred more quickly in the first study when the grafts were morselized twice. The differences with fresh-frozen grafts became significant after three impactions, while in the present work, grafts were morselized once and significant differences were seen only after 10–50 up to 150 impactions. Passage through the bone mill may even embrittle the bone before impaction.

Compaction by as many as 150 blows may be unusual in clinical work, but improved the mechanical result. The maximal stiffness value obtained in the undefatted fresh-frozen control group after 150 impactions (175 Mpa) was reached after only 70 impactions with the freeze-dried irradiated graft. This means that the use of freeze-dried irradiated bone grafts may facilitate the surgical procedure by reducing the number of hammer shocks need for a stable reconstruction. Nevertheless, if implant stability is the first goal, the impaction procedure must be done energetically, until the impactors can not be driven further into the bone. Surgeons must bear in mind that the in vitro impaction conditions may be better than the clinical ones. When freeze-dried irradiated bone morsels are used, 50% more graft will be needed to fill in the same defect than with use of fresh-frozen bone.

Bone graft reconstruction remodeling is disputed. Some steps in the preparation of the grafts are thought to favor osteoconduction (Thoren et al. 1995), while others may change the metabolic properties (protein denaturation) and greater compactness may slow the remodeling (Tägil and Aspenberg 1998). Incomplete bone regeneration can not be avoided. Therefore, surgeons must concentrate on the mechanical stability of the construct (Linder 2000).

We conclude that the brittleness of freeze-dried irradiated bone, which is due to the loss of its ability to absorb energy in a plastic way after final irradiation, increases the compactness and stiffness of the morselized grafts. Freeze-dried irradiated morselized bone was mechanically stronger than fresh-frozen bones that had been defatted, but not irradiated, and those that had not been defatted as regards stiffness. Because of its ability to become impacted faster, a stiffer graft mantle can be obtained. Washing bone with saline alone or treating bone with a solvent-detergent, but no irradiation, did not give a similar mechanical advantage and did not impact better than fresh-frozen undefatted bone in our model.

No competing interests declared.

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