Impaction bone grafting with freeze-dried irradiated bone. Part II. Changes in stiffness and compactness of morselized grafts

Experiments in cadavers

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ABSTRACT In the technique of impaction bone grafting, implant stability depends on the mechanical properties of the impacted morselized grafts. Although the procedure is usually performed with fresh-frozen femoral heads, there is still some concern about their supply and safety. Bone processing is a potential solution, but the mechanical properties of this material during and after impaction need to be determined.

We used 6 osteoarthrotic femoral heads to prepare two paired batches of morselized bone. One batch was morselized and frozen. The other batch was chemically treated, morselized, freeze-dried and then gamma-irradiated. We impacted 18 samples from each batch in a contained cylinder. Freeze-dried bone grafts were tested after 30 minutes of rehydration. The changes in the compactness and stiffness of the material were monitored during the impaction. The compaction of the freeze-dried bone was faster than that of their fresh-frozen control. The maximal stiffness reached by both materials was the same (55 MPa), but the freeze-dried grafts required three to four times fewer impactions to achieve that stiffness. After 3, 10 and 50 impactions the freeze-dried bone was stiffer than the fresh-frozen bone. As it is easier to impact, the freeze-dried bone may be mechanically more efficient than the fresh-frozen bone in surgical conditions. Moreover, the processed bone meets the highest safety standards, as regards the risk of disease transmission.

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Impaction bone grafting is usually done with morselized fresh-frozen femoral heads. The use of this grafting material carries a potential, but well-established, risk of disease transmission (Tomford 1995). This risk is greater when the bone grafting material comes from several donors. A hip revision with impaction bone grafting usually requires two to three femoral heads (Galea et al. 1998, Henman and Finlayson 2000). Moreover, most bone banks are currently finding it difficult to provide fresh-frozen femoral heads (Norman-Taylor and Villar 1997).

Freeze-dried irradiated bone has been used for selected indications in orthopedics and is now a time-honored material (Delloye et al. 1987, Fabry 1991, Cornu et al. 1995). At present, the preparation of these grafts includes removal of marrow and cells, treatment with solvent-detergent, prion inactivation, freeze-drying and gamma irradiation. These steps have a cumulative effect in eliminating the risk of disease transmission in such a way that the whole procedure produces a very safe human grafting material.

Owing to the change in the mechanical properties of bone by freeze-drying and, most of all, by the final gamma irradiation (Pelker et al. 1984, Anderson et al. 1992), it has been used cautiously in clinical practice. Irradiated freeze-dried bone from femoral heads is known to be less strong, less stiff and significantly more brittle than the fresh-frozen controls when tested in compression (Cornu et al. 2000).

In a recent study, we impacted various types of fresh-frozen morselized grafts in a small chamber and demonstrated the harmful effect of cartilage inclusions (Bavadekar et al. 2001). In the present investigation, using the same procedure, we compared the irradiated freeze-dried bone with fresh-frozen bone to determine the changes in stiffness and compactness of both materials during impaction.

Material and methods

Morselized graft preparation

Our experimental protocol is based on a previously described method (Bavadekar et al. 2001). 6 human femoral heads (from 6 patients, 2 women) were procured at the time of primary hip arthroplasty for osteoarthrosis, and stored at –80 °C. The median age of the donors was 72 (53–75) years. The femoral heads were shaved off of all their soft tissues (articular cartilage remnants and synovium), while the cortical neck was retained. The heads were then divided into two halves with a band saw. The half heads of the fresh-frozen group containing their original marrow fat were stored frozen at –80 °C. The other halves were subjected to the following processing. They were thoroughly washed under a jet of deionized water to remove bone marrow and blood cells. Lipids were extracted by a 1:1 (v/v) chloroform-methanol solution renewed 3 times for at least 2 days and rinsed with methanol and water (Delloye et al. 1987). After denaturation of the bone proteins (prion inactivating procedure) they were rinsed again in deionized water for 24 hours. The half heads from both groups were then morselized twice when wet, without any defatting step, with the small rasps of the Noviomagus bone mill (Spierings, Nijmegen, NL). Two batches of grafts were created. The morselized grafts from the processed group were placed in open vials, freeze-dried for 48–72 hours (temperature of the condenser –80 °C, temperature of the chamber –30 °C, working vacuum 1×10^{-4} mm of Hg) and irradiated at a minimal irradiation dose of 25 kGy (maximum 30 kGy, source of irradiation Cobalt $_{60}$). After freeze-drying and irradiation, the residual moisture of the bone was calculated by gravimetry at 2.5% of dry material (Sartorius MA 30, Goettingen, Germany).

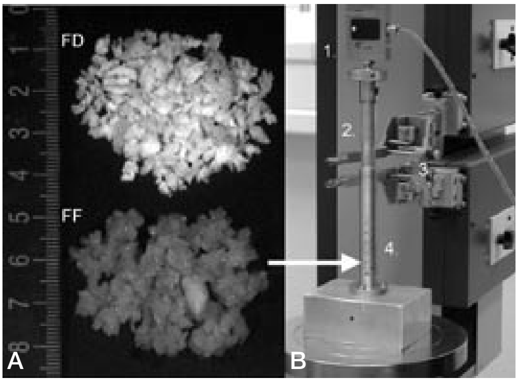


Figure 1. A. Typical appearance of the two types of morselized grafts (left). B. A sample was poured into a cylinder (right) with small vents (4). Using a dropping mass (not shown) the impactor (2) telescoped the grafts in the cylinder. The forces were measured with a 2 kN load cell (1) in a testing machine, and displacement of the impactor gliding inside the cylinder was measured with an extensometer (3).

The fresh-frozen grafts lose much fat, marrow and water during such processing. After weighing the samples at the beginning and the end of the process, we calculated that a sample of 5 g of fresh-frozen morselized graft weighs only 1.75 g after the procedure (Figure 1A).

18 randomly selected samples of both types of grafts (5 g and 1.75 g, respectively) were prepared for the mechanical test. In this experimental design, the fresh-frozen bone grafts were the controls and the freeze-dried bone the test group.

Mechanical testing

For mechanical testing, a sample of morselized graft was put into an aluminum tube (inner diameter 14.6 mm) and compacted by a solid cylinder telescoping freely in the tube over the grafts. The aluminium tube had micro-holes for draining saline and fat from the graft during compaction. The impaction itself was produced by a mass of 455 g falling from a height of 1 m on the solid cylinder.

The fresh-frozen morselized grafts 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 in the cylinder and tested. The impaction 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 and its stiffness. The height was measured with a digital caliper as the distance between the top of the tube and a fixed point on the impactor. The compressive stiffness (or Emod, MPa) of the impacted grafts was measured by placing the experimental setting in a testing machine (Zwick model Z50/TH3A, Zwick GmbH, Ulm, Germany). The upper plate of the machine gently compressed the impacting material at a speed of 0.5 mm/min. 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 (Figure 1B). To avoid excessive compression of the grafts during the measurement, we limited the test to 80 N of force (0.5 MPa) or 0.3 mm of displacement. Stiffness was calculated as the slope of the curve between 60% and 98% of the maximal load (the linear part of the curve). After reaching this limit, the cylinder of grafts was immediately unloaded. Knowing the time-dependent mechanical properties of morselized grafts (creep and recoil) (Ullmark and Nilsson 1999), we could standardize the testing conditions for each sample. The height and stiffness measurements were made in one minute between the impactions. Since the samples were used for density measurements, we made 18 measurements 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 third parameter was the change in the apparent density of the impacted material. To monitor the densitometric changes, we interrupted the procedure at different stages of the impaction. The impacted cylinders were gently expressed from the aluminum cylinder, placed in plastic tubes and immediately frozen on dry ice. Therefore, of the 18 samples in each group, 4 were impacted until the third impaction, 4 until the tenth impaction, 4 until the fiftieth and 6 until the final 150th impaction. The frozen impacted specimens were scanned with a pQCT (peripheral quantitative computed tomography machine, model XCT Research SA+, Stratec, Pforzheim, Germany). The density value is expressed in g/cm^3.

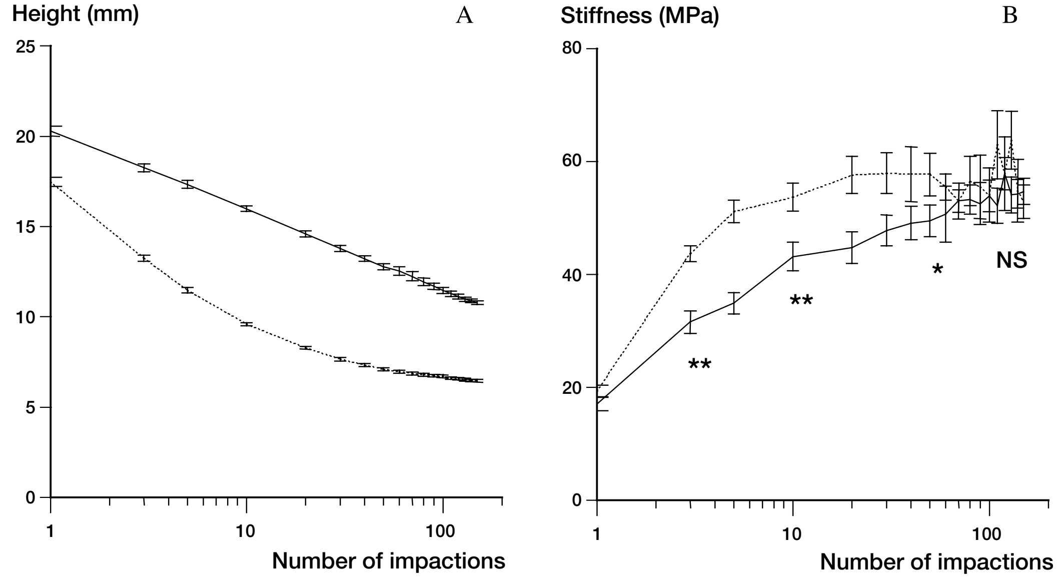
Statistics

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

Results

In the cylinder, the initial height of both types of samples (at 0 impactions, not shown in the log-scaled graph) was the same (26 mm). However, we found considerable differences between the two types of grafts during the impaction (Figure 2A). At 150 impactions, the freeze-dried graft layer deformed to about one third of its initial height while the fresh-frozen control reached half of its initial height. At every step of the impaction procedure the freeze-dried samples reached higher compactness ($p < 0.001$).

The patterns of rise in stiffness of the two types of grafts showed similarities and differences (Figure 2B). Both the freeze-dried and the fresh-frozen grafts reached a final mean modulus of about 55 MPa after 150 impactions. The freeze-dried bone showed a rapid rise in stiffness reaching a stable and maximal value of 55 MPa at 20 impactions. In contrast, the fresh-frozen morselized bone showed a slow, but steady rise in the modulus with successive impactions. At the seventieth impaction, the value was the same as that of the freeze-dried bone. The ANOVA with repeated measures showed that the rise in stiffness was significantly faster up to 3 impactions ($p < 0.001$) up to 10 impactions ($p < 0.001$) and up to 50 impactions ($p = 0.005$) but not when the whole curve was considered up to 150 impactions. This confirms that the rise in stiffness is faster with freeze-dried grafts.



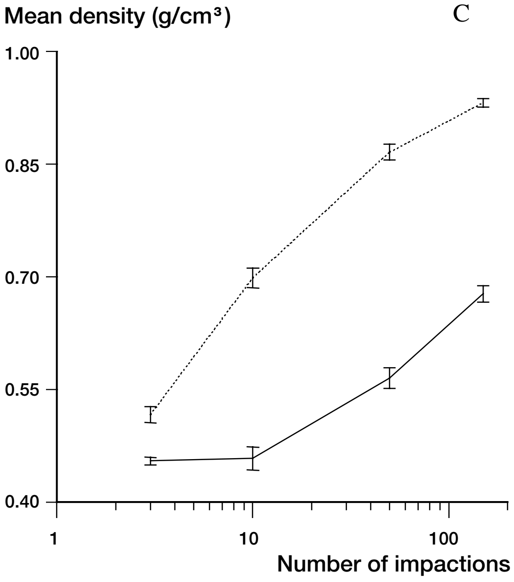


Figure 2. Freeze-dried grafts (dotted line) compacted much faster than the fresh-frozen bone grafts (solid line), as shown by the fast reduction in height (A); therefore the rise in stiffness was faster (B). The stiffness was significantly higher up to 3, 10 and 50 impactions (**: $p < 0.001$; *: $p < 0.005$), but not up to 150 impactions. If 70 impactions are applied to the grafts, the modulus is identical (about 55 MPa). The change in the density (C) confirmed the measurements of compactness. Note that the number of impactions is shown on a log scale. The line represents the mean and the bars the standard error of this mean.

In both groups, the density increased during impactions (Figure 2C). The mean density of fresh-frozen morselized grafts rose from a value of 0.45 g/cm^3 at 3 impactions to 0.65 g/cm^3 at 150 impactions. In contrast, the freeze-dried morselized grafts showed a rapid rise in density from 0.5 g/cm^3 at 3 impactions to a final value of 0.95 g/cm^3 at 150 impactions. At 3, 10, 50 and 150 impactions, the density was significantly higher in the freeze-dried bone ($p = 0.005$, $p < 0.001$, $p < 0.001$ and $p < 0.001$. respectively).

Discussion

In a previous study, we compared the static compressive properties of fresh-frozen and freeze-dried bone from femoral heads (Cornu et al. 2000). Similarly, samples were rehydrated for 30 min. in normal saline before the mechanical testing. We found that the treatment induced reductions in the strength (42%), in stiffness (21%) and, particularly in work to failure (embrittlement of the bone) (75%). On the other hand, the in-vitro cyclic loading of cadaver femurs showed that the reconstructions done with the freeze-dried bone were paradoxically more stable than those done with fresh-frozen bone. The present study partly answers the question: How can a softer material give a stiffer reconstruction?

We know that the stiffness of the graft layer depends mainly on the compactness obtained with repeated impactions (Bavadekar et al. 2001). The freeze-dried bone was simply impacted much faster than the fresh-frozen control (Figure 2). Several explanations may account for this. First, on impacting the grafts, the stress is applied to the material at high speed. In such conditions, the flow of liquids may play an important role (Carter and Hayes 1977) and hence, the replacement of viscous bone marrow by saline in the freeze-dried bone may accelerate the compaction of the grafts (Kärrholm et al. 1999). Secondly, the embrittlement due to bone processing (Cornu et al. 2000) may be of value in impaction bone grafting. The more brittle, the faster the compaction. Therefore, even if each morsel of graft has a 21% lower modulus, a faster rise in compactness causes a faster rise in stiffness (Figure 2B).

We tried hard to ensure an unbiased comparison of the two types of morselized allografts. In both groups, the grafts came from the same 6 femoral heads. Therefore, the morsels being mixed had lower between-sample variability which enhanced the differences between the two groups. In the freeze-dried group, the weight of the material in one sample was adjusted to account for the loss of marrow, fat and water during the processing. Thus, the same amount of bone material was placed in the cylinder (as shown by the identical initial height before the first drop of the mass, 26 mm).

The higher stability in part I seems to be explained by the present data. When impacted with the same energy, the freeze-dried grafts probably created a layer that had a higher density (compactness) and therefore a higher modulus. To fill a femoral defect, the operator will use more bone (2–2.5 heads) on the freeze-dried side than on the fresh-frozen side (1.5 heads). This ratio corresponds to the measurements of height in the second experiment (Figure 2A). Consequently, although we could not measure the local mantle density in the first experiment, we believe that the graft packing was better. The difference in the inducible displacement and subsidence may be due to differences in compactness/density of the grafts.

The main advantage of the bone processing (and freeze-drying) is the striking reduction in the risk of disease transmission which occurs thanks to several steps in the inactivating treatments. Moreover, the present data indicate that it may also improve the surgical procedure. From the practicaly point of view, three to five times fewer hammer shocks are needed to impact the freeze-dried bone correctly and obtain the same modulus as with fresh-frozen bone. In the operating room, this may spare surgical time and reduce the risk of cortical femoral fractures that remain one of the complications of the impaction procedure (Slooff et al. 1996, Leopold et al. 2000, Pekkarinen et al. 2000). Dispensed as ready to use and stored packed at room temperatures, the morselized freeze-dried grafts have clinical implications in further saving surgical time, commonly lost when morselizing the fresh-frozen femoral heads. Finally, as there is no quarantine period for the freeze-dried grafts (European Association of Musculoskeletal Transplantation (EAMST) and European association of Tissue Banks (EATB) 1997), such grafts can be made more readily available than fresh-frozen femoral heads.

On the other hand, the use of freeze-dried bone may also have various disadvantages. First, as the material is easier to impact, the surgeon may use more grafts. However, as regards the improved packing, a more stable reconstruction can be expected. Secondly, more studies are needed to determine whether the freeze-dried bone is more or less osteoconductive than its fresh-frozen control. The defatting and washing promotes osteoconductivity (Thoren et al. 1995), the freeze drying reduces the graft antigenicity (Friedlaender et al. 1984). On the other hand, the higher compactness may reduce the speed of remodeling (Tägil and Aspenberg 1998).

We conclude that the freeze-dried irradiated cancellous bone is mechanically better than its fresh-frozen controls. Because of its ability to become impacted faster, a stiffer graft mantle can be obtained. The highest safety standards do not necessarily occur at the expense of future implant stability.

No competing interests declared.

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