

The Biomechanical Properties of Deep Freezing and Freeze Drying Bones and Their Biomechanical Changes after in-vivo Allograft

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This study measured the physical properties in bending of the rat femur and compression of the rat first tail vertebra subjected to deep freezing at -80°C for 2 weeks, 6 weeks, 12 weeks and freeze drying. This study also measured the mechanical changes after in vivo allograft of fresh bone, deep freezing(2, 6, 12weeks) and freeze drying. Analysis for deep freezing groups showed a mean 7.2% decrease in bending strength and 11.0% decrease in compressive strength when compared with the control group, but there was no statistical difference in the duration of deep freezing. The groups of in vivo graft after deep freezing showed 23.1% and 22.2% decrease in bending and compressive strength. There was no statistical difference in the duration of deep freezing. The freeze drying group showed a 97% decrease in bending strength and no significant difference in compressive strength. The group of in vivo graft after freeze drying showed a 30.1% and a 41.3 % decrease in bending and compressive strength. The above results suggested that there would be some mechanical limitation in using freeze dried graft for supporting implants.

Key Words: Bone, allograft, freezing, freeze drying, biomechanics

Bone allografts are being used more frequently in the management of a wide spectrum of orthopaedic conditions. Their successful application is predicted on sound knowledge of their biological properties and of their capacity to withstand the stresses to which they will be subjected. Several alternative methods are currently used to preserve and store specimens until they are required for implantation. There has been no study on the mechanical change in the duration of the deep freezing and the change after in vivo allograft. The duration and extent of re-

hydration has been suggested by Bright and Burstein(1978). In 1993, Conrad *et al.* reported that rehydration of freeze dried grafts adversely affect the graft strength and stiffness. If rehydration does have a deleterious effect on the strength of grafts, these grafts might deteriorate biomechanically as the grafts become more completely rehydrated after implantation. This study measured the physical properties in bending of the rat femur and compression of the rat first tail vertebra subjected to deep freezing at -80 centigrade for 2 weeks, 6 weeks, 12 weeks and freeze drying. This study also measured the mechanical changes after in vivo allograft of fresh bone, deep freezing(2, 6, 12weeks) and freeze drying.

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MATERIAL AND METHODS

The bones used in this study were obtained

from adult Sprague-Dawley rats weighing 350 ± 5 gr. The femurs and tail vertebrae were harvested at sacrifice, and all soft tissue was removed by sharp dissection. The following experimental groups were prepared: fresh bone serving as control, deep freezing bone for 2, 6 and 12 weeks, freeze drying bone, in vivo allograft of fresh bone, allograft of deep freezing (2, 6 and 12 weeks) and allograft of freeze drying bone. All specimens were divided into 10 groups randomly and 10 specimens were included in each group. The strength of each test group was expressed as a percentage of the control group. The deep freezing group was frozen at -80 centigrade (ULT, 286, Revco Scientific Inc., North Carolina, USA). The freeze drying group were frozen first at -80 centigrade for one week and then lyophilized at -40 Centigrade and 0.02 mmHg for 24 hours (75540J-200099 Labconco Company, Kansas, USA) to a residual moisture content of less than 3%. The gravimetric method (Komender 1976) was used for moisture content analysis. In vivo allograft was done on the subcutaneous tissue of the thigh of the rat and 2 weeks later, the strength of each group of graft bones was tested. The freeze dried specimens were rehydrated in normal saline for 24 hours before mounting and testing. The other bones were brought in a bath of normal saline at room temperature before testing. Femurs were tested for failure in bending and vertebrae were tested for failure in compression (model 4501, Instron corporation, Boston, USA) at a constant deformation rate 0.5 mm/sec. For the bending test, special metal blocks were designed for holding the femur. Each end of the femur was inserted into the holes of the space block and mounted in quick setting polyester resin to ensure a firm fixation. A spacing block insured that a standard testing distance of 1.5 cm of bone was maintained between the mounting blocks and that force was applied just at the middle point of each of the specimens. For compression testing, each vertebra had its base embedded in a thin layer of resin to allow it to remain upright during the testing procedure. Compressive force was applied directly to the superior endplates. The resulting load deformation

curves were digitalized and analyzed by computer. The statistical analysis was done with Mann-Whitney U test.

RESULTS

The values for each biomechanical parameter for each group were averaged and the mean and standard deviations computed. The strength of each tested group was expressed as a percentage of control group. In the bending strength, 2 weeks freezing group showed 6.8% decrease, 6 weeks freezing group 9.6% decrease and 12 weeks freezing group 5.2% decrease (all data $p < 0.05$) but there was no statistical difference in the duration of freezing ($p > 0.05$). In the compressive strength, 2 weeks freezing group showed 10.0% decrease, 6 weeks freezing group 11.4% decrease and 12 weeks freezing 11.4% decrease (all data $p < 0.05$) but there was no statistical difference in the duration of freezing group ($p > 0.05$). Analysis for deep freezing groups showed mean 7.2% decrease in bending strength and 11.0 % decrease in compressive strength when compared with control group ($p < 0.05$). The group of in vivo graft (fresh bone) showed 17.6% and 20.2% decrease in bending and compressive strength respectively ($p < 0.05$). The graft group after 2 weeks freezing showed 22.7% decrease in bending strength, 23.2% decrease in compressive strength ($p < 0.05$). After 6 weeks freezing, the graft group showed a 24.8% decrease in bending strength, 21.8% decrease in compressive strength ($p < 0.05$). After 12 weeks freezing, the graft group showed a 21.7% decrease in bending strength, 21.9% decrease in compressive strength ($p < 0.05$). However, there was no statistical difference in the duration of freezing ($p > 0.05$). So, on average, the groups of in vivo graft after deep freezing showed 23.1% and 22.2% decrease in bending and compressive strength ($p < 0.05$). However, there was no statistical difference in the duration of deep freezing ($p > 0.05$). The freeze drying group showed 9.7% decrease in bending strength ($p < 0.05$) and no significant difference in compressive strength ($p > 0.05$). The group of in vivo

Table 1. Bending strength tested in femora

| Group | Bending strength(N) | Percentage |
|---------------------|---------------------|------------|
| Control | 140.03±7.67 | 100 |
| Deep freeze 2 wks | 130.49±7.95 | 93.2 |
| Deep freeze 6 wks | 126.64±5.98 | 90.4 |
| Deep freeze 12 wks | 132.87±5.89 | 94.8 |
| Fresh Graft | 115.51±7.47 | 82.4 |
| 2 wks Freeze graft | 108.25±6.55 | 77.3 |
| 6 wks Freeze graft | 105.43±5.01 | 75.2 |
| 12 wks Freeze graft | 109.67±6.15 | 78.3 |
| Freeze drying | 126.57±9.43 | 90.3 |
| Fr-Dry graft | 97.97±5.46 | 69.9 |

(Fr: freezing, All data $p < 0.05$ compared with control)

Table 2. Compressive strength tested in vertebrae

| Group | Compressive strength(N) | Percentage |
|---------------------|-------------------------|------------|
| Control | 163.89±5.77 | 100 |
| Deep freeze 2 wks | 147.64±8.52 | 90.0 |
| Deep freeze 6 wks | 145.36±9.60 | 88.6 |
| Deep freeze 12 wks | 144.80±5.21 | 88.3 |
| Fresh Graft | 130.86±4.01 | 79.8 |
| 2 wks Freeze graft | 126.00±5.11 | 76.8 |
| 6 wks Freeze graft | 128.30±4.33 | 78.2 |
| 12 wks Freeze graft | 123.04±4.65 | 78.1 |
| Freeze drying | 162.25±2.71 | 98.9 |
| Fr-Dry graft | 96.35±7.11 | 58.7 |

(Fr: freezing, All data $p < 0.05$ compared with control except*)

*: $p > 0.05$)

graft after freeze drying showed 30.1% and 41.3% decrease in bending and compressive strength($p < 0.05$).

DISCUSSION

The use of various forms of bone grafts to

replace or supplement diseased portions of the skeleton has become a frequent occurrence in clinical practice. The successful use of these tissues is dependent on a multiplicity of varying biological, physiological and biomechanical factors, each of which influences the result of transplantation(Horowitz and Friedlaender 1987). In considering the physical properties of these transplantable tissues, the individual factors that may influence their structure must be analyzed. Freezing and freeze drying have been shown to decrease the antigen response to allograft. Freezing and freeze drying also preserve the bone and allows longterm storage. However, the biomechanical properties of allograft implants are affected by the method of preservation. It is generally accepted that freezing bones to -25 centigrade will result in little if any alteration in their physical properties(Sedlin 1965). However, enzymatic degradation is not completely arrested at this temperature(Friedlaender and Mankin 1981). Regarding the influence of deep freezing on the mechanical properties, slight variations of 10 to 20% increase or decrease have been noted in the failure of strength(Komender 1976, Pelker *et al.* 1982). Pelker *et al.*(1984) reported that deep freezing did not adversely affect mechanical strength. In this study deep freezing groups showed a mean 7.2% decrease in bending strength and 11.0% decrease in compressive strength when compared with the control group, but there was no statistical difference in the duration of deep freezing. The group of in vivo graft(fresh bone) showed 17.6 % and 20.2 % decrease in bending and compressive strength respectively. The groups of in vivo graft after deep freezing each showed a 23.1% and 22.2% decrease in bending and compressive strength. However, there was no statistical difference in the duration of deep freezing. Previous investigations of the effects of freeze drying on bone have evaluated different animal model with various methods. Pelker *et al.* (1984) reported no significant decrease in the compressive strength of freeze dried bone in rat vertebrae. In contrast, Triantaphyllou *et al.* (1975) found a significant reduction in bending strength and the elastic modulus of freeze dried bovine bone. Malinin

et al. (1989) found no significant decrease in the bending strength of human femoral cross sections following freeze drying. Recently Conrad *et al.* (1993) studied the effects of the rehydration of freeze dried human cancellous bone and found that unrehydrated grafts appeared to be both stronger and stiffer than the rehydrated counterparts. In this study the freeze drying group showed a 9.7% decrease in bending strength and no significant difference in compressive strength. The group of in vivo graft after freeze drying showed 30.1% and 41.3% decreases in bending and compressive strength. Rapid freezing of specimens resulting in the rapid expansion of trapped liquid with subsequent crack propagation can lead to weakening of the tissues (Pelker and Friedlaender 1987). The rehydration of a freeze dried graft weakens perhaps as a result of some biochemical alteration in collagen cross linkage. In vivo grafts become highly rehydrated and necrotic bone is weakened by resorptive phase after 2 weeks of graft. Our results suggested that deep freezing caused reduction of bony strength but the duration of freezing was not a factor and the freeze drying caused marked reduction of the mechanical strength after in vivo graft. In conclusion, there would be some mechanical limitation in using freeze dried graft for structural implantation. Further investigation of the freeze drying and rehydration techniques and their respective biomechanical effects in bone grafts may assist in the handling of freeze dried structural grafts.

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