THE EFFECTS OF FREEZE-DRYING ON THE MECHANICAL PROPERTIES OF HUMAN CORTICAL BONE

INTRODUCTION
Allogeneic bone is the most commonly grafted tissue.\(^1\) Gifts are required for a variety of orthopaedic procedures and the demand for this reconstructive material can only be met by tissue banking. Methods of preservation routinely used include freezing and freeze-drying.\(^2\) Freezing is generally accepted as a method of preservation that does not alter the physical properties of bone,\(^3\) but frozen bone grafts are expensive to store and transport. Reports on the effects of freeze-drying on the mechanical properties of bone, however, are conflicting.\(^4\) As such studies are used to assess the suitability of freeze-dried bone for grafting,\(^5\) it is important to have a range of comparable results from independent investigations. However, there are relatively few available that simultaneously investigate several properties of human bone in standard ways. This lack of comprehensive information leads to the objectives of our study; to determine the effects of Tissue Bank freeze-drying on the mechanical properties of human cortical bone.

METHODS

Paired human femora were obtained from four U.K. Tissue Banks (A, B, C and D), each having their own protocol for freeze-drying. The tissue was unsuitable for clinical use and informed consent had been obtained for it to be used for research. Tissue and fat were removed from the bones at their respective tissue bank. Each femur was cut just below the lesser trochanter and just above the adductor tubercle, and the resulting hollow cylinder of cortical bone was cut transversely into 3 cylinders producing cortical rings approximately 50 mm in length. One of the pair was frozen at -80°C as a control and the other was freeze-dried at the tissue bank. Defrosted and rehydrated cortical rings were cut longitudinally into prisms (2x4 mm cross section), under constant irrigation using a diamond-bladed bandsaw (Exakt). This procedure was repeated in identical positions on the contralateral ring. To ensure any remaining flaws were inherent, specimens were ground using carborundum paper of progressively finer grades.

Approximately half of the specimens were tested wet in quasi-static four-point bending using an Instron table top testing machine and others in four-point impact (Hounsfield Plastic Impact Tester). Two more pairs of bones were obtained from Tissue bank D to investigate further the effects seen on bones freeze-dried according to this bank’s protocol. The first pair was treated exactly as before. However, one of the bones from the second pair was dried (20°C) in a vacuum desiccator using silica gel as a moisture trap, and the other was frozen as a control.

RESULTS

Table 1: Mechanical properties of human cortical bone (tissue bank, age, sex) that has been either freeze-dried or desiccated (Exp) or frozen (Con).

<table>
<thead>
<tr>
<th>Bone</th>
<th>Young’s Modulus (GPa)</th>
<th>Bending Strength (MPa)</th>
<th>Work of Fracture (kJ m(^{-2}))</th>
<th>Impact Energy (kJ m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 17F</td>
<td>15.8 (11)</td>
<td>13.6 (11)</td>
<td>184.8 (12)</td>
<td>214.9 (10)</td>
</tr>
<tr>
<td>B 49F</td>
<td>16.6 (6)</td>
<td>13.3 (6)</td>
<td>177.2 (6)</td>
<td>13.1 (7)</td>
</tr>
<tr>
<td>C 23F</td>
<td>15.6 (5)</td>
<td>14.1 (5)</td>
<td>165.6 (5)</td>
<td>14.3 (6)</td>
</tr>
<tr>
<td>D 43M</td>
<td>16.7 (16)</td>
<td>14.9 (16)</td>
<td>176.0 (16)</td>
<td>15.1 (16)</td>
</tr>
<tr>
<td>D 67F</td>
<td>13.5 (7)</td>
<td>12.9 (7)</td>
<td>134.8 (7)</td>
<td>15.3 (6)</td>
</tr>
<tr>
<td>D 24F</td>
<td>16.1 (7)</td>
<td>13.7 (7)</td>
<td>176.3 (7)</td>
<td>22.5 (7)</td>
</tr>
</tbody>
</table>

Table 1 shows the data obtained for six pairs of bone, five having been split into freeze-dried specimens with frozen controls, the sixth (D 24F), being room temperature desiccated specimens with frozen controls. Values given are mean values for (\(^\#\)) number of samples (dependent on the thickness of the cortex for each individual donor). The results of a Student’s t-test carried out on the differences between the dried specimens and their paired frozen controls are shown; *\(p<0.05\), **\(p<0.005\), ***\(p<0.0001\).

DISCUSSION

The amount of variation seen in the effects of freeze-drying on the mechanical properties of bone tested in bending is consistent with that seen in the literature. This seems to be due to the lack of standardization of the actual freeze-drying process of bone for allograft purposes. Only two out of the four bone banks, (three pairs of femora tested) used the same protocol for freeze-drying; C and D. The effect of this was a similar apparent toughening shown by the three bones freeze-dried in this way. Freeze-drying is a method of preservation that should produce a dry bone suitable for room temperature storage which, after rehydration, should be minimally altered, mechanically or otherwise. For bones freeze-dried at banks C and D a tougher bone was being produced. This had not been reported in previous bending studies for freeze-dried bone, although has been noted before following room temperature drying\(^6\) and so prompted the final set of experiments using room temperature desiccation.

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REFERENCES


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\(\approx\) One or more of the authors have received something of value from a commercial or other party related directly or indirectly to the subject of my presentation.

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