We examined solvent-dried, gamma-irradiated (SD-R) allografts and fresh-frozen (FF) allografts mechanically and morphologically. Before transplantation, FF grafts were more than six times stronger than SD-R grafts. After four weeks, the tensile strength was about the same in both groups. At 24 weeks only collagen fibrils of small diameter were observed in the SD-R grafts while in FF grafts fibrils of small and intermediate diameter were seen. Clinically, we suggest that SD-R grafts could be used as a favourable alternative to FF grafts if care was taken regarding their initial mechanical weakness.

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Materials and Methods

We used 16-week-old male Lewis rats (LEW/crj, Charles River, Japan) as donors and Wistar rats (Crj:Wistar, Charles River, Japan) as recipients.

We harvested the medial half of the patellar tendon with a tibial bone block from both knees of 24 Lewis rats. The bone block was 4 mm wide and 8 mm long and the tendon graft 2.5 mm wide and 8 mm long. These grafts were randomly allocated to two groups, FF or SD-R. The latter were suspended in 3% hydrogen peroxide, and then dried with organic acetone followed by gamma irradiation (2.8 Mrads). This treatment has been confirmed to inactivate human immunodeficiency virus. The FF grafts were stored at −80°C for two to five weeks before transplantation.

At the time of transplantation FF grafts were thawed at room temperature and SD-R grafts were reconstituted in saline for 30 minutes. Each recipient rat was operated on under general anaesthesia by intravenous injection of ketamine to create defects in the medial half of the patellar tendon and the tibia of both legs which were then replaced with a graft. The tibial bone block was fixed with a 0.7 mm Kirschner wire and the proximal and lateral ends of the graft were sutured with 6-0 nylon thread. After transplantation, each animal was allowed free activity in the cage.

Mechanical studies. Groups of three rats were killed at 4, 8, 12 and 24 weeks after transplantation and the transplanted patella-graft-tibia complexes were harvested. Four grafts of each group before transplantation were also prepared for evaluation.

The cross-sectional areas of the grafts were measured, using the method of Butler et al, before transplantation and at the time of killing. To make the aspect ratio appropriate (1:5), 1 mm wide specimens were used for testing of tensile strain-to-failure. Just before tensile testing, the cross-sectional areas of the specimens were measured.

The patella-graft-tibia complex was clamped and mounted in a materials testing machine (Model AO75001; Tokyo-koki Seizosho Ltd, Tokyo, Japan). After preconditioning, the specimens were tested to failure at a crosshead speed of 10 mm per minute. The deformation was analysed in a video dimension analyser (Percept Scope C3160; Hamamatsu Photonics, Hamamatsu, Japan) to obtain stress and...
strain curves. Tensile strength, tangent modulus and strain at failure were calculated. Maximum load was estimated by multiplying the tensile strength by the cross-sectional area.

Statistical assessment was performed using an analysis of variance (ANOVA) to compare the two groups at each of the five time points. Subsequent comparisons were performed using Fisher’s method to detect significant differences (p < 0.05).

**Histological studies.** We examined histologically the part of each transplanted graft which had not been used for mechanical testing. Fresh patellar tendons from 16-week-old male Lewis rats were also examined. Half of each specimen was fixed in 10% neutral formaldehyde, embedded in paraffin for light microscopy, cut into sections 3 μm thick, and stained with haematoxylin and eosin. Cellularity, nuclear morphology, fibre alignment, and interfibrillar spaces were evaluated by light microscopy. Nuclear morphology was classified as linear, spindle, oblong, or round, according to the grading system reported by Rougraff et al.\textsuperscript{10}

The other half of each specimen was used for transmission electron microscopy. Each was fixed in 2.5% glutaraldehyde and 2% paraformaldehyde and embedded in Epon 812. Ultrathin sections, cut at 90° to the collagen fibril axis and stained with uranyl acetate and lead citrate, were examined under a transmission electron microscope (JEOL 1200EX; JEOL, Tokyo, Japan). Five suitable fields from each specimen were photographed at a magnification of 20 000. The diameter of the collagen fibrils was expressed as the percentage area of the fibrils.

**Results**

**Mechanical evaluation.** The mean (± SEM) cross-sectional area of the specimens is shown in Figure 1. In both groups the cross-sectional area of the grafts doubled after transplantation with no significant differences between the two

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**Table I.** Mechanical properties (mean ± SEM) of solvent-dried and gamma-irradiated allografts and fresh-frozen allografts

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Group</th>
<th>Number</th>
<th>Tensile strength (MPa)</th>
<th>Tangent modulus (MPa)</th>
<th>Strain at failure* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>SD-R</td>
<td>4</td>
<td>8.4 ± 0.9†</td>
<td>120.2 ± 32.4‡</td>
<td>12.1 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>FF</td>
<td>4</td>
<td>55.3 ± 4.0</td>
<td>420.5 ± 45.2</td>
<td>19.5 ± 1.9</td>
</tr>
<tr>
<td>4</td>
<td>SD-R</td>
<td>4</td>
<td>12.5 ± 3.0</td>
<td>136.2 ± 32.8</td>
<td>14.7 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>FF</td>
<td>4</td>
<td>14.2 ± 1.9</td>
<td>150.8 ± 21.7</td>
<td>17.5 ± 3.1</td>
</tr>
<tr>
<td>8</td>
<td>SD-R</td>
<td>5</td>
<td>24.3 ± 4.1§</td>
<td>168.8 ± 26.0</td>
<td>15.2 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>FF</td>
<td>5</td>
<td>10.1 ± 1.1</td>
<td>108.5 ± 34.9</td>
<td>12.0 ± 3.0</td>
</tr>
<tr>
<td>12</td>
<td>SD-R</td>
<td>3</td>
<td>22.7 ± 2.2</td>
<td>185.8 ± 40.5</td>
<td>16.3 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>FF</td>
<td>4</td>
<td>17.3 ± 1.1</td>
<td>171.6 ± 37.7</td>
<td>15.3 ± 4.1</td>
</tr>
<tr>
<td>24</td>
<td>SD-R</td>
<td>4</td>
<td>26.7 ± 3.9</td>
<td>223.6 ± 24.6</td>
<td>19.2 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>FF</td>
<td>4</td>
<td>26.9 ± 4.7</td>
<td>238.0 ± 41.6</td>
<td>17.9 ± 4.5</td>
</tr>
</tbody>
</table>

* not significantly different from the value for FF grafts at 0 weeks (p > 0.05)
† significantly different from the value for FF grafts at the same period (p < 0.0001)
‡ significantly different from the value for FF grafts at the same period (p < 0.01)
§ significantly different from the value for FF grafts at the same period (p < 0.05)
Fig. 3
The mean (± SEM) tangent modulus of the patellar tendon grafts after transplantation.

Fig. 4
The mean (± SEM) maximum load of the patellar tendon grafts after transplantation.

Fig. 5
Photomicrographs of normal patellar tendon and SD-R and FF patellar tendon grafts before transplantation and at 0, 4, 12 and 24 weeks (haematoxylin and eosin ×75).
For testing of tensile/strain-to-failure, we analysed 20 of 28 specimens in the SD-R group (71%) and 21 of 28 specimens in the FF group (75%) which had failed at the mid-substance. Six specimens which had failed at their proximal suture site and nine which had failed at their distal bone insertions were excluded from the mechanical evaluation.

Details of the results are given in Table I and the changes in tensile strength with time in Figure 2. Before implantation, the tensile strength of the FF grafts was significantly larger than that of the SD-R grafts. After transplantation, that of FF grafts markedly decreased so that at four weeks the tensile strength of the two groups was equal. At eight weeks, the tensile strength of the SD-R grafts increased significantly compared with that of FF grafts; at 24 weeks it fell to about the same level as that of the FF groups.

The change in the tangent modulus with time followed a similar pattern (Fig. 3). At zero time the tangent modulus of SD-R grafts was significantly smaller than that of FF grafts. After transplantation there was no significant difference at any time point.

The strain at failure of each group ranged from 12% to 20% with no remarkable trends in terms of the group and period after transplantation (Table I).

The maximum load of both types of graft increased with time (Fig. 4), and at 24 weeks reached about 80% of that of the FF graft at zero time, but there were no significant differences in this value.

**Histological findings.** Before transplantation, both types of graft differed considerably from the normal patellar tendon (Fig. 5). The SD-R grafts had no cellularity, a larger amplitude and pitch of crimp and wider interfibrillar spaces than normal tendons and the FF grafts also showed less cellularity and had wider interfibrillar spaces. At four weeks after transplantation there were more cells in both grafts than in normal control tendons. The alignment of the collagen fibres of the FF grafts was more normal than that of SD-R grafts.
of the SD-R grafts. The cells of the SD-R grafts were mainly oblong while those of the FF grafts were predominantly spindle-shaped or linear. At 12 weeks, in both types of graft, the collagen fibres were more longitudinally aligned and cellularity had decreased but remained greater than in normal control tendons. At 24 weeks, the alignment and the crimp pattern of the collagen fibres had become normal in both groups, but there was still a larger number of cells, especially in the SD-R grafts. The cells in both types of graft were mainly spindle-shaped or linear.

During the experimental period neither type of graft showed signs of immunological rejection such as lymphocytic invasion. **Collagen fibril diameter.** Compared with control tendons, collagen fibrils in FF grafts before transplantation were similar in diameter but had wider interfibrillar spaces. Those in SD-R grafts at zero time had much larger diameters than those of normal tendons and were polygonal (Fig. 6).

After transplantation, both types of graft differed from the normal patellar tendon in terms of the collagen fibril profile (Figs 6 and 7). In the normal patellar tendon fibrils of intermediate diameter (150 and 200 nm) occupied 71% of the area and those of small diameter (50 and 100 nm) only 9% of the area. At four weeks after transplantation, the small and intermediate fibrils occupied a larger area in the FF grafts while only small fibrils were seen in the SD-R grafts. The bimodal pattern in the FF grafts was still seen at 24 weeks, but the percentage area of small fibrils was greater than that at four weeks. In SD-R grafts only small fibrils were found throughout the entire experimental period.

**Discussion**

Ligament reconstruction, using allografts, is commonly performed and the reported success rates are high. Previous reports have shown that after transplantation, the tensile strength of FF grafts markedly decreased and then gradually increased and we have confirmed this finding. By contrast, SD-R grafts showed little decrease in tensile strength after transplantation.

Although there was little difference in the recovery of the mechanical properties between the two groups after transplantation, their collagen fibril profile was totally different. The collagen fibrils of FF grafts had a bimodal pattern while SD-R grafts had only fibrils of small diameter suggesting that their remodelling process was different. In general, small fibrils in transplanted tendon grafts are thought to be newly synthesised. Clinical studies have
shown that successfully reconstructed anterior cruciate ligaments using FF allografts have only fibrils of small diameter. SD-R grafts may therefore act as scaffolds which are completely replaced with newly synthesised collagen fibrils.

Most animal models of ligament reconstruction cause overstress to the grafts during the early postoperative period. In our experimental model, half of the patellar tendon was left intact to avoid such overstress. Consequently, the initial mechanical weakness of SD-R grafts did not result in failure. In clinical use, if care is taken after operation and a well-controlled rehabilitation programme is implemented, it is possible that SD-R grafts could be a favourable alternative to FF grafts for ligament reconstruction.

Our experimental model, however, has also some weaknesses in regard to clinical relevance. It is an extra-articular reconstruction and only half the tendon is replaced. The use of SD-R grafts for reconstruction of the human anterior cruciate ligament, which requires total replacement in an intra-articular environment, cannot therefore be directly extrapolated from this study.

Conclusion. Solvent-drying and gamma irradiation are good methods of preservation for the sterilisation and storage of allogeneic soft tissue, but have adverse effects on the initial mechanical properties of allografts. Our study has shown that this mechanical weakness of solvent-dried and gamma-irradiated allografts disappears at an early stage. With enough care and well-controlled rehabilitation they may be suitable for clinical application.

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References