Despite widespread use of radiofrequency (RF) shrinkage, there have been no animal studies on the effects of post-operative immobilisation on the histological properties of the shrunken tissue. We have therefore examined the role of post-operative immobilisation after RF shrinkage with special emphasis on the histological properties of collagenous tissue.

One patellar tendon of 66 New Zealand White rabbits was shrunk. Six rabbits were killed immediately after the operation. Twenty rabbits were not immobilised, 20 were immobilised for three weeks and 20 for six weeks. Fibroblasts, collagen and vascular quality and density were evaluated on sections, stained by haematoxylin and eosin.

Nine weeks after operation the histological properties were inferior to those of the contralateral control tendons. Shrunken tendons did not return to normal at any time after operation irrespective of whether the animals had been immobilised or not. All the parameters improved significantly between zero and three weeks after operation. Immobilised tendons tended to have a better and faster recovery.

Careful rehabilitation is imperative after RF shrinkage. Immobilisation aids recovery of the histological properties. Our findings in this animal model support a period of immobilisation of more than three weeks.
every 5 mm between the patellar and the proximal tibial wire to allow consistent application of energy.

The RF energy was delivered to the tendon using an RF generator (Vapr II; Mitek Division Ethicon, Norderstedt, Germany) at a temperature setting of 70˚C and a power setting of 40W, coupled with a bipolar, temperature-controlled probe (Vapr TC; Mitek Division Ethicon). In order to allow the probe to be used under saline a plastic cup of 10 cm in diameter equipped with a rubber base was placed over the knee. A slit in the rubber base of the cup allowed the patella, patellar tendon and proximal tibia to protrude into the cup. This was then filled with saline and a consistent medial-to-lateral grid pattern was used moving the probe from proximal to distal at a velocity of approximately 1 to 2 mm/sec with the parallel passes spaced approximately 5 mm apart. The incision was closed with simple interrupted 3-0 vicryl sutures (Vicryl; Ethicon).

After operation, the rabbits were divided into different groups according to the immobilisation plan. Six were killed immediately after the operation. Twenty were allowed normal cage activity without any immobilisation. Ten of these were killed after three weeks, and ten after nine weeks. Twenty were immobilised in a cast for three weeks; of which ten were killed directly after removal of the cast at three weeks and ten were allowed normal cage activity for another three weeks without a cast and were killed six weeks after surgery. Finally, 20 were immobilised in a cast for six weeks; ten of these were killed directly after removal of the cast at six weeks, and ten were allowed normal cage activity for another three weeks without a cast and were killed nine weeks after surgery.

The patella, patellar tendon and proximal tibia were disarticulated as a unit from the remainder of the limb in both hindlimbs in all rabbits. The left leg served as a control. The skin and subcutaneous tissue were carefully removed and the tendons were wrapped in saline swabs and frozen at -80°C. After thawing of the patella-patellar-tendon-tibial complexes specimens 0.6 mm x 0.3 mm x 0.3 mm in size were cut from each patellar tendon close to the tibial insertion of the tendon. The specimens were fixed in neutral buffered 7% formalin. They were embedded in paraffin and sections 5 µm thick were cut in the sagittal plane. These were stained with haematoxylin and eosin and examined by light microscopy and polarised light microscopy. Two specimens from the left limbs of each group were selected at random for use as controls.

In order to evaluate the effect of RF energy on collagenous tissue, we used a subjective scoring system according to Hecht et al. All the tissue sections were evaluated and graded in a blinded manner. The scoring system for the quality of fibroblasts and collagen is shown in Table I. The grading of quality and the density of vessels was also performed according to Hecht et al (Table II).

**Statistical analysis.** The scores were reported as the mean value with SD. A Mann-Whitney U test was used to evaluate differences between different groups. The level of significance was set at 5%.

**Results**

**Fibroblasts.** The fibroblasts in the control sections had a typical spindle-shaped appearance with no alterations of cell nuclei and cell orientation. The subjective mean score for the fibroblasts in the control tendons was 1.08 (SD 0.19).

Immediately after operation the fibroblasts showed marked changes with nuclear karyorrhexis (damage of the cell nucleus) and pyknosis of the nuclei. The mean score

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**Table I.** The subjective scoring system for the quality of fibroblasts and collagen according to Hecht et al

<table>
<thead>
<tr>
<th>Score</th>
<th>Fibroblasts</th>
<th>Collagen (light and polarised light microscopy)</th>
<th>Hyalinisation</th>
<th>Unorganised bundles</th>
<th>Laminar organised, normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>100</td>
<td>Dead cells, nuclear karyorhexis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>75</td>
<td>Proliferative, active cells</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>Normal, spindle-shaped cells</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>Normal, spindle-shaped cells</td>
<td>75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>Normal, spindle-shaped cells</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>Normal, spindle-shaped cells</td>
<td>75</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>Normal, spindle-shaped cells</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>Normal, spindle-shaped cells</td>
<td>25</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>Normal, spindle-shaped cells</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table II.** Subjective scoring system of the vascular quality and density according to Hecht et al

<table>
<thead>
<tr>
<th>Score</th>
<th>Vascular quality</th>
<th>Vascular density</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal appearance of vessels</td>
<td>Normal amount</td>
</tr>
<tr>
<td>2</td>
<td>Plump endothelial cells, capillary sprouting</td>
<td>Increased amount</td>
</tr>
<tr>
<td>3</td>
<td>Occluded vessels, pyknotic endothelial cells</td>
<td>Increased amount of dead cells</td>
</tr>
</tbody>
</table>
was 6.75 (SD 0.69). Three weeks after operation the sections of the non-immobilised and immobilised limbs still showed changes in the morphology of the fibroblasts with polymorphic nuclei. The mean score for immobilised tendons was 2.81 (SD 1.51) and for non-immobilised tendons 3.55 (SD 1.21). This difference was not significant (p = 0.37).

Six weeks after operation the appearance of the fibroblasts was more similar to that of the control sections and the fibroblasts were more spindle-shaped. The mean score for immobilised tendons was 2.4 (SD 1.07). That for tendons which had been immobilised during the first three weeks and had a subsequent period of three weeks without immobilisation was 2.85 (SD 0.7). The difference was not significant (p = 0.22).

Nine weeks after treatment the fibroblasts were similar to the spindle-shaped cells of the control sections. Despite the hypercellularity the cell orientation was almost normal. The mean score for non-immobilised tendons was 2.75 (SD 0.68). That for tendons which had been immobilised during the first six weeks was 3.00 (SD 0.82). The difference was not significant (p = 0.43).

The change in the scores over the first three weeks showed a significant recovery of the fibroblasts in both the immobilised and the non-immobilised groups (p = 0.002). The further improvement in the scores was not statistically significant. At no time did the treated fibroblasts reach the score of the control sections irrespective of whether they had been immobilised or not. Nine weeks after operation the score of both groups had still increased compared with control tendons (p < 0.001) (Fig. 1).

**Light-microscopic findings.** The control sections had a normal appearance of wavy fibrous collagen bundles with a mean score of 1.08 (SD 0.19).

Immediately after the operation there was a loss of the normal fine fibrillar collagen structure and hyalination led to a mean score of 6.91 (SD 0.58).

Three weeks after operation the hyalination of the collagen structure had disappeared almost completely. Fine, but mainly unorganised, fibrillar bundles of collagen were visible. Sections of immobilised tendons showed some areas of organised collagen and had a mean score of 2.93 (SD 1.29). Non-immobilised tendons had completely unorganised collagen bundles and a mean score of 4.35 (SD 1.13). There was an apparent difference between immobilised and non-immobilised tendons but it was not significant (p = 0.054).

Six weeks after the operation, tissue from immobilised tendons had almost the same appearance as at three weeks. The mean score was 2.85 (SD 1.4). The sections of the tendons which had been immobilised during the first three weeks revealed some areas of organised collagen bundles and had a mean score of 3.00 (SD 0.67). The difference between the groups was not significant (p = 0.731).

Nine weeks after operation both groups had areas of wavy organised collagen bundles next to some minor unorganised areas. The collagen bundles were more distinct and the tissue had a more homogenous appearance. The mean score for non-immobilised tendons was 3.10 (SD 0.84), and that for tendons which had been immobilised during the first six weeks was 3.05 (SD 1.03). The difference was not significant (p = 0.877).

The change in the scores for collagen quality throughout the study period revealed a significant improvement in the collagen in the first three weeks for both the immobilised and the non-immobilised tendons (p = 0.002). The further recovery of the tissue was not statistically significant. The treated collagen did not recover sufficiently to reach the score of the control sections irrespective of whether it had been immobilised or not. Nine weeks after operation the mean score of both groups was still statistically worse compared with that of the control tissue (p < 0.001; Figs. 2 and 3; Table III).

**Polarised microscopic findings.** The control sections had a normal, wavy birefringence. Since all sections had this appearance, the mean score for the control sections was 3.10 (SD 0.84), and that for tendons which had been immobilised during the first six weeks was 3.05 (SD 1.03). The difference was not significant (p = 0.877).

The change in the scores for collagen quality throughout the study period revealed a significant improvement in the collagen in the first three weeks for both the immobilised and the non-immobilised tendons (p = 0.002). The further recovery of the tissue was not statistically significant. The treated collagen did not recover sufficiently to reach the score of the control sections irrespective of whether it had been immobilised or not. Nine weeks after operation the mean score of both groups was still statistically worse compared with that of the control tissue (p < 0.001; Figs. 2 and 3; Table III).
Three weeks after operation the sections of immobilised and non-immobilised tendons had almost complete loss of birefringence with unorganised collagen bundles. The mean score for the immobilised tendons was 3.13 (SD 1.89), and that for non-immobilised tendons 4.69 (SD 1.35). The difference between these groups was not significant (p = 0.117).

At six weeks the tissue of the immobilised limbs showed the beginning of reorganisation of collagen bundles and the intensity of birefringence had improved. The mean score
was 3.40 (SD 1.58). The tendons which had been immobi-
lised for the first three weeks only had the same appearance
as at three weeks. Their mean score was 4.50 (SD 0.71). The
difference between completely immobilised and partially
immobilised limbs was not statistically significant at six
weeks (p = 0.12).

By nine weeks both groups showed an improvement in
collagen organisation. The mean score for tendons which
had been immobilised during the first six weeks was 3.60
(SD 1.26). The mean score for non-immobilised limbs was
3.40 (SD 1.27). The difference was not significant (p =
0.69).

The time-related change in the scores for collagen quality
showed a significant improvement of the organisation of
collagen in the first three weeks. The difference for immo-
bilised tendons was statistically significant (p = 0.005), as
was that for non-immobilised tendons (p = 0.049). The fur-
ther recovery of the tissue was not statistically significant.
At no time did the score of the treated samples reach that of
the control sections irrespective of whether they had been
immobilised or not. The mean score of both groups was
still statistically worse at nine weeks compared with that of
the control tissue (p < 0.001) (Fig. 4; Table III).

**Vascular quality and vascular density.** In the control sec-
tions the endothelial cells of the vessels showed a normal
spindle-shaped appearance of their nuclei and a low vascu-
lar density. The mean score for vascular quality and density
was 1.00 (SD 0.00).

Immediately after the operation the tissue showed a
marked alteration of the vascular quality with thrombosed
vessels and endothelial cells necrosis. The mean score for
vascular quality was 2.83 (SD 0.41). The density of the ves-
sels was unchanged compared with the control sections but
the vessels were seriously damaged and thus the mean score
for vascular density was 2.83 (SD 0.41).

Three weeks after operation the sections showed almost
complete regeneration of the endothelial cells. The tissue of
the non-immobilised limbs had enlarged endothelial cells
and a mean score for vascular quality of 1.95 (SD 0.28). The
vessels of the immobilised tendons had smaller endothelial
cells and a mean score of 1.38 (SD 0.44). The difference in vascular quality between the groups was statistically significant at three weeks (p = 0.009). Both groups showed a marked increase in vascular density with hypervascularity. The mean score for vascular density of the non-immobilised tendons was 1.95 (SD 0.37); and that for the immobilised tendons was 1.63 (SD 0.44). This difference was not significant (p = 0.07).

At six weeks the vascular quality of both groups was approximately the same as that in the group immobilised for three weeks. The mean score of the immobilised tissue was 1.5 (SD 0.41) and that for tendons which had been immobilised during the first three weeks 1.45 (SD 0.37). The difference was not significant (p = 0.77). Both groups still had increased vascular density compared with the control sections. The mean score for vascular density in the immobilised limbs was 1.75 (SD 0.42) and that for tendons which had been immobilised during the first three weeks was 1.6 (SD 0.31). This difference was not significant (p = 0.22).

By nine weeks the vascular quality had improved further. The score of the tissue which had been immobilised during the first six weeks was 1.45 (SD 0.28) and that for the non-immobilised limbs was 1.30 (SD 0.34). This difference was not significant (p = 0.25). The score for vascular density was the same in both groups at nine weeks: 1.75 (immobilised, SD 0.42; non-immobilised, SD 0.35).

The changes in the score for vascular quality during the study showed a significant improvement in the first three weeks (p = 0.002 for non-immobilised limbs and p = 0.003 for immobilised limbs). At three weeks the vessels of the immobilised tissue had a significantly better quality than those of non-immobilised limbs (p = 0.009). The further recovery of the vessels was not statistically significant for immobilised tendons. The vascular quality for non-immobilised tissue improved between three weeks and nine weeks (p < 0.001).

The vascular density showed a similar improvement in the first three weeks (p = 0.002 for non-immobilised limbs and p = 0.003 for immobilised limbs). The further improvement of the score for vascular density was not significant. At no time did the scores for the vascular quality or the density of the shrunk tissue reach those of the control sections irrespective of whether the limbs had been immobilised or not. At nine weeks the mean score for both groups was worse compared with that for the control tissue (p < 0.001) (Fig. 5, Table IV).

**Discussion**

Thermal shrinkage of collagenous tissue has generated wide interest recently. In 1997 Pullin et al.12 found in a canine model significant inflammation, necrosis and hypercellularity six weeks after laser-assisted shrinkage. Also Schaef er et al.6 described in a rabbit model a generalised fibroblastic response which was characterised by a marked increase in cellularity and vascularity after eight weeks. In 1998 Hecht et al.22 found cell infiltration, collagen fusion and pyknosis of fibroblasts seven days after treatment in a sheep model. They showed that the area and depth of the thermal damage were influenced by the power setting of the RF generator. In another study Hecht et al.10 examined changes in a sheep model after monopolar RF for two, six and 12 weeks. They also described early hyalinisation of collagen and cell necrosis followed by active tissue repair. After 12 weeks they found laminar collagenous tissue and a normal synovial membrane.10 Hayashi et al.21 described the biological response to laser treatment in a sheep model with a post-operative follow-up of three, seven, 14, 30, 60, 90 and 180 days. They also found collagen hyalinisation and cell necrosis and found the tissue to be histologically normal after 60 days.

Our results are comparable to the findings of Schaef er et al.6 and Pullin et al.12 and are in contrast with those of Hayashi et al.21 and Hecht et al.22 who found a histologically normal appearance at six weeks and full recovery by 60 days.

Since this is the first in vivo animal study of the influence of post-operative immobilisation our results in the immobilised animals are not comparable with those of previous studies. It is not clear why the tissue did not recover completely despite immobilisation but one possible explanation is that the duration of immobilisation was too short. Recently published clinical studies have described the
results of laser- and RF-induced capsular shrinkage in patients with impingement, multidirectional instability and capsular laxity.\textsuperscript{15-18} The patients were immobilised for two to three weeks and had a high rate of satisfactory results after a mean follow-up of two years. These promising clinical mid-term results are in contrast to our histological findings. It may be that we are unaware of the factors responsible for the clinical success of thermal treatment. Induction of fibrosis, thickening of the joint capsule or thermal denervation have been suggested as mechanisms.\textsuperscript{21}

In a study on the role of immobilisation on post-operative tissue length Pötzl et al\textsuperscript{11} found that significant lengthening could not be prevented even with immobilisation. These observations correlate with our histological results which indicate that a period of six weeks of post-operative immobilisation is too short for complete recovery of the tissue. The results of our study must be interpreted with caution. The patellar tendon of the rabbit is not the ideal model of the human shoulder. The difference between tendons and capsular ligaments should be taken into account. The structure and the collagen content of tendons and ligaments are not the same. Another important difference is that tendons are attached to muscles and even during immobilisation the muscles may pull on the tendons. It is difficult to compare the immobilisation of a rabbit’s knee in a cast with the immobilisation of a human shoulder in a sling, but the differences between the non-immobilised and immobilised animals showed that immobilisation had a significant effect. The patellar tendon tissue in our model was normal, but pathological features of the joint capsule in glenohumeral instability have not been well defined.\textsuperscript{24} We were also unable to control the energy applied in the \textit{in vivo} study as precisely as in \textit{in vitro} studies.\textsuperscript{21}

In conclusion our results demonstrate the considerable importance of careful post-operative rehabilitation after RF-induced shrinkage of collagenous tissue. In order to prevent serious damage shrunk tissue should be protected from normal physiological loads. The histological properties of shrunk tissue are markedly altered. This decrease in the tissue quality because of the thermal treatment is demonstrated by the post-operative lengthening of the treated tissue and the inferior biomechanical properties.\textsuperscript{6,9,11} Although there was a considerable improvement, in this animal model the treated tendons did not recover a normal histological appearance even under immobilising conditions.

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