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ORIGINAL ARTICLE

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Histological and biomechanical observations of the rabbit patellar tendon after removal of its central one-third

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Abstract Using 35 Japanese white rabbits, a study was made of tissue regeneration and the mechanical properties of the patellar tendon after removal of its central one-third. After removal of the central one-third of the patellar tendon on one side, in experiment 1 the strength of the entire patellar tendon including the regenerated tissue was compared with that of the patellar tendon on the opposite side with the central one-third removed at the time of killing, and in experiment 2 the strength of only the regenerated tissue was compared with that of the patellar tendon on the opposite side with two-thirds of the medial and lateral sides removed at the time of death. In experiment 1, the maximum load showed no significant difference between the operated side and the control. In one-half of the cases, the strength of the operated side including the regenerated tissue was weak, suggesting weakening of the patellar tendon on the residual bilateral sides. In experiment 2, the maximum load of the regenerated tissue was significantly lower than that of the control, the former being 25% of the latter even at 6 months. Histologically, the characteristics of the cells and collagen fibers gradually approached those of normal tissue, but the crimp pattern of the collagen fibers and fibrils was evidently smaller than that of the control. These results indicate that regenerated tissue was still mechanically weak and immature at 6 months.

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Introduction

In anterior cruciate ligament (ACL) reconstruction by autograft, a number of tissues have been employed as donor, but since the report of Noyes et al. [11] on the strength of the patellar tendon, use of the central one-third of the patellar tendon has become the gold standard. However, there seems to be no agreement on the recovery of the mechanical strength of the patellar tendon after removal of this one-third. As for observations made following this removal, in the report of Berg [4] concerning follow-up at 8 months postoperatively, hypertrophy of the patellar tendon was demonstrated by magnetic resonance imaging (MRI), and histologically, it was replaced by almost normal tendinous tissue composed of collagen fibers. Coupens et al. [8] carried out a postoperative MRI follow-up of the patellar tendon in 20 cases ranging from 6 weeks to 18 months and observed an increase in the thickness of the patellar tendon during the entire follow-up period. Cabaud et al. [7] removed the medial one-third of the canine patellar tendon and observed 8 months postoperatively that the strength of the operated side was superior to that of the control side [10]. However, on the other hand, Bonamo et al. [5] reported that, when the central one-third of the patellar tendon was used as the donor, rupture of the patellar tendon was observed 4 months and 8 months thereafter in two cases. Burks et al. [6] in their study on the mechanical characteristics of the canine patellar tendon following removal of the central one-third, observed a failure load of 70% and 60% compared with the control after 3 months and 6 months, respectively. Jackson et al. [9] in their experiment using goat patellar tendon noted a remarkable increase in the cross-sectional area 6 months thereafter, but a drop in failure load to 50%.

The present study was conducted with the aim of elucidating how the patellar tendon defect is regenerated, whether the regenerated tissue is mature tissue similar to normal patellar tendon, and whether it can re-acquire the strength equivalent to that of a normal patellar tendon.

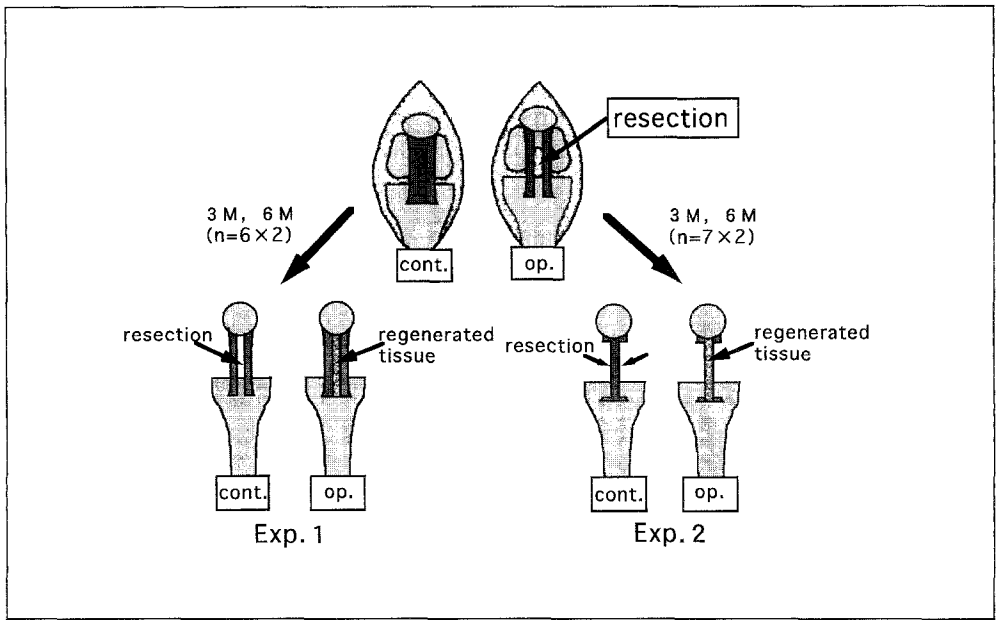


Fig. 1 Study method: after removal of the central one-third of the patellar tendon on one side, in experiment 1 the strength of the entire patellar tendon including the regenerated tissue was compared with that of the patellar tendon on the opposite side which had its central one-third removed at the time of killing; in experiment 2 the strength of only the regenerated tissue was compared with that of the patellar tendon on the opposite side with two-thirds of the medial and lateral sides removed at the time of killing

Materials and methods

In the present study, 35 male Japanese white rabbits weighing about 2500 g were used. The central one-third of the patellar tendon of the hind limb on one side was removed and, as a control, only separation of the patellar tendon from the subcutaneous tissue was done on the opposite side. The animals were killed 1, 3, and 6 months thereafter and the regeneration process of the defect observed, together with a mechanical test being conducted at 3 and 6 months. In experiment 1, the maximum load and the stiffness of the entire patellar tendon including the regenerated tissue of the operated side were measured, while in the control, the central one-third was removed at the time of killing, and then the maximum load and the stiffness of what remained of the patellar tendon were measured. In experiment 2, the maximum load and the stiffness of only the central one-third of the regenerated tissue on the operated side were measured, and in the control only the central one-third of the intact patellar tendon was maintained and measured. In experiment 1, six rabbits each were used at 3 months and 6 months, and in experiment 2, seven rabbits each were used (Fig. 1). Histological specimens were prepared for all the rabbits after the mechanical test and ones of intact tissue not subjected to mechanical test were also prepared for three rabbits each in the 1-month group, 3-month group, and 6-month group.

Surgical technique

For anesthesia, sodium pentobarbital was intravenously administered (35 mg/kg). From the anteromedial approach, the central one-third of the patellar tendon of the hind limb on one side was resected together with a part of the patella and tibia, and on both sides of the resected part, three stitches were made in the margins with 6-0 nylon thread to permit subsequent observation. Only the skin was sutured and closed. On the opposite side, using the same approach, only the anterior surface of the patellar tendon was separated from the subcutaneous tissue as control. The animals were not immobilized, being permitted to move freely within the cage. The rabbits were killed 1, 3, and 6 months thereafter, and patella-patellar tendon-tibia complex was removed. Scar-like connective tissue of the patellar tendon surface and the surrounding soft tissue were resected. Following extirpation, the specimens were immersed in physiological saline at room temperature in order to prevent dehydration.

Macroscopic observation

At the time of extirpation, the condition of the resected site and the patellar tendon were grossly observed, and the length and thickness of the patellar tendon together with the width of the resected site were measured with a caliper [12]. For this measurement, the patella and patellar tendon were separated from the surrounding tissue, and after fixing the tibia, a load of 100 g was applied to the patella.

Mechanical test

The patella and the shaft of the tibia were embedded in a rectangular aluminum tube filled with resin, and in addition, the exposed tibia was covered with resin. They were then hardened in physiological saline to prevent any effect of the polymerization heat on the tendon tissue. Aluminum tubes were fixed with clamps so that the patella and the tibia would be aligned. Using the Shimazu universal testing instrument, a mechanical test was carried out at a strain rate of 50 mm/min [13]. With the data thus obtained, load-deformation curves were prepared with the Autograph AGS 1000A system, and as two parameters which would represent the structural property of the tendon, maximum load and linear stiffness were determined. Linear stiffness is the load per deformation and is represented as the linear slope of the load-deformation curve. The mechanical test was conducted within 6 h after removal of the tissues from the animal.

Histological observation

After fixing in 10% buffered formalin and embedding in paraffin, the specimens were stained with hematoxylin and eosin. The morphology and number of cells and the arrangement of collagen fibers were recorded. The number of cells per visual field at × 160 magnification was counted, and the mean of each of the three random visual fields was compared. Using hematoxylin and eosin-stained specimens, the crimp pattern of collagen fibers was observed with a simple polarizing apparatus. The crimp amplitude and crimp period were measured on × 480 magnification film, and the results were computed from the mean of the three sites. Crimp is a feature of both tendons and ligaments and presents a regular sinusoidal pattern in the matrix. Amplitude and periodicity of the crimp appear to be structure-specific features. Crimp provides a

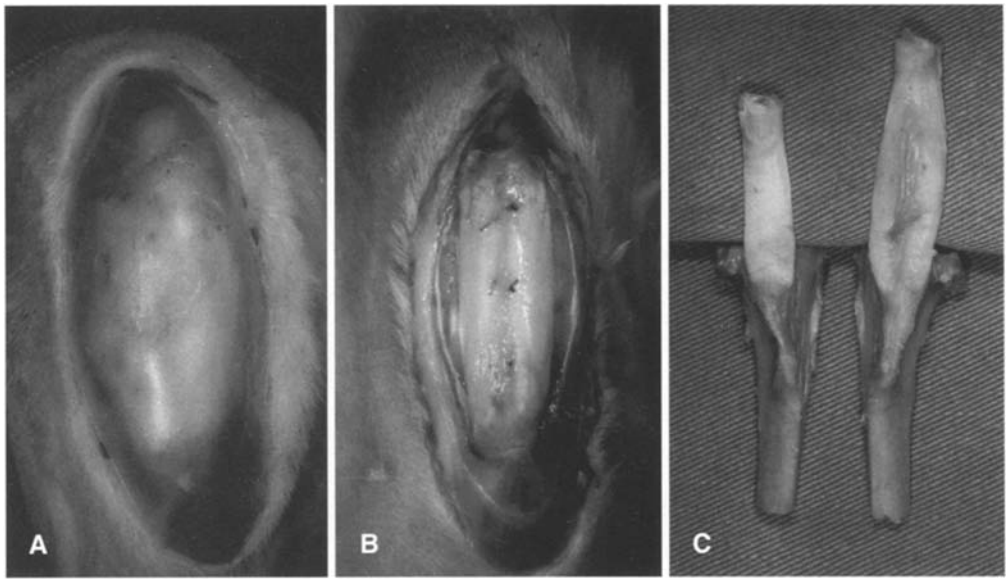


Fig. 2 Macroscopic appearance 6 months postoperatively: **A** thickening of the connective tissue on the anterior aspect of the patellar tendon; **B** appearance after removal of the connective tissue; **C** elongation of the patellar tendon

mechanism for the control of tension and acts as a "shock absorber" along the length of the tissue.

In line with the standard preparation procedure, transverse thin sections were fixed in 2.5% glutaraldehyde and embedded in epon. Using specimens stained with toluidine blue, the distribution of the collagen fibrils by diameter size and the ratio of the area occupied by collagen fibrils [1] were evaluated. Using the cross-sectional view, collagen fibrils were classified per 25 nm in diameter, and their number was determined on × 63000 magnification film. From the mean of five random visual fields, their respective ratios were computed. The area occupied by collagen fibrils was measured by an automatic image analyzer Nexus Cube.

Results

Macroscopic findings

At 1 month, regenerated tissue could be observed at the resected site, but its amount was small. At 3 months, the tissue quantity had increased, and the surface of the patellar tendon was covered with scar-like connective tissue. At 6 months, the connective tissue had thickened, rendering the nylon thread scarcely visible. When this surface layer of connective tissue was removed, white semi-transparent regenerated tissue could be readily distinguished from the patellar tendon even at 6 months (Fig. 2). For a mean resection of 2.9 mm, the width of the resected site was 2.8 ± 1.6 mm in the 3-month group and 2.4 ± 1.2 mm in the 6-month group. Though there was some range in values for each specimen, the repair of the defect site was not achieved by hypertrophy of the remaining patellar tendon but by replacement through regenerated tissue. The length of the patellar tendon was 23.9 ± 3.5 mm on the operated side of the 3-month group, with that of the control being 18.5 ± 1.6 mm. The length of the operated side of the 6-month group was 23.1 ± 4.3 mm, with that of the control being 18.8 ± 1.6 mm, demonstrating a significant elongation in both the 3-month and 6-month groups. The

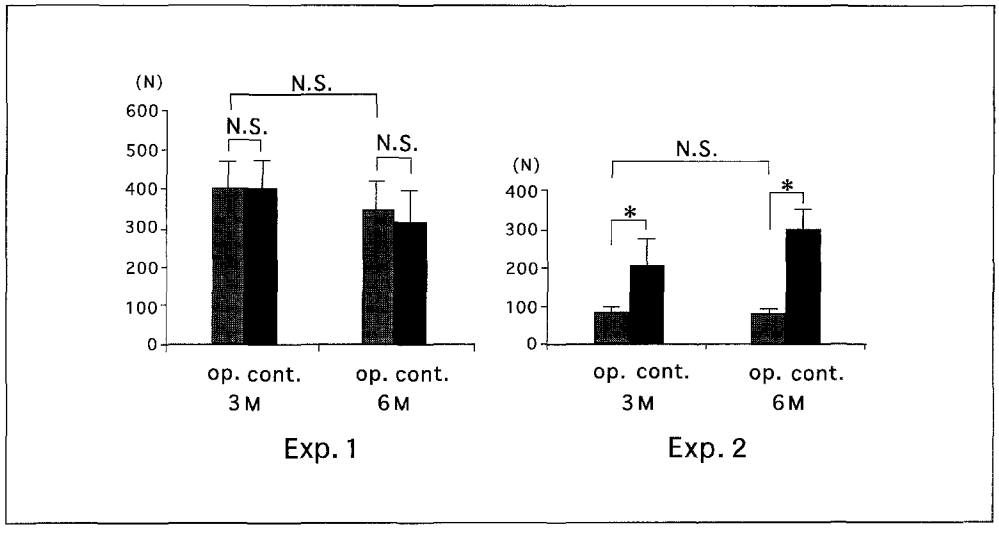


Fig. 3 Maximum load of the patellar tendon. In experiment 1, a significant difference was not observed in maximum load between the operated side and control, but in experiment 2, the maximum load of the regenerated tissue was significantly lower than that of the control (* $P < 0.05$)

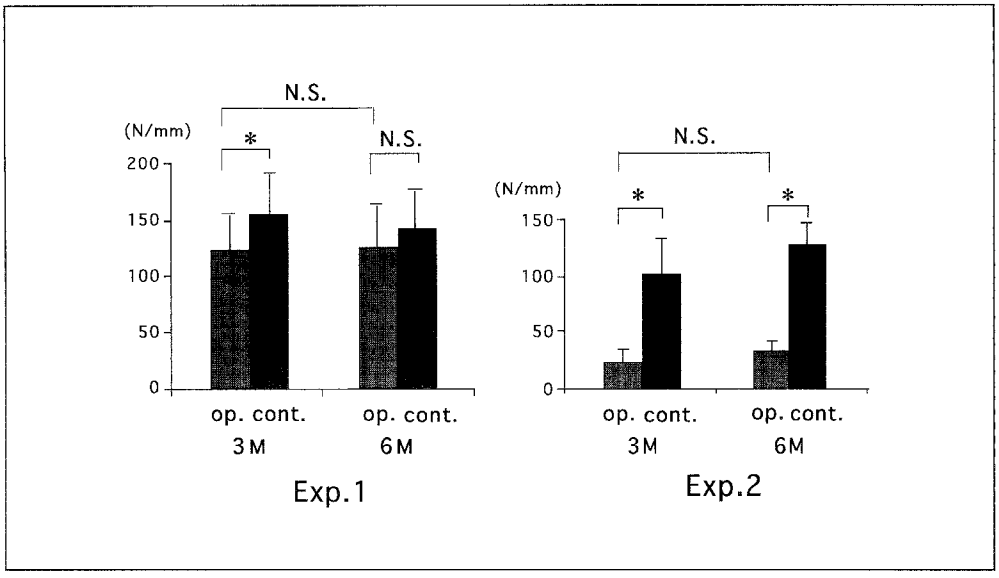


Fig. 4 Linear stiffness of the patellar tendon. In experiment 1, the stiffness of the operated side was lower than that of the control, and a significant difference was observed in the 3-month group (* $P < 0.05$). In experiment 2, the stiffness of the regenerated tissue was significantly lower than that of the control (* $P < 0.05$)

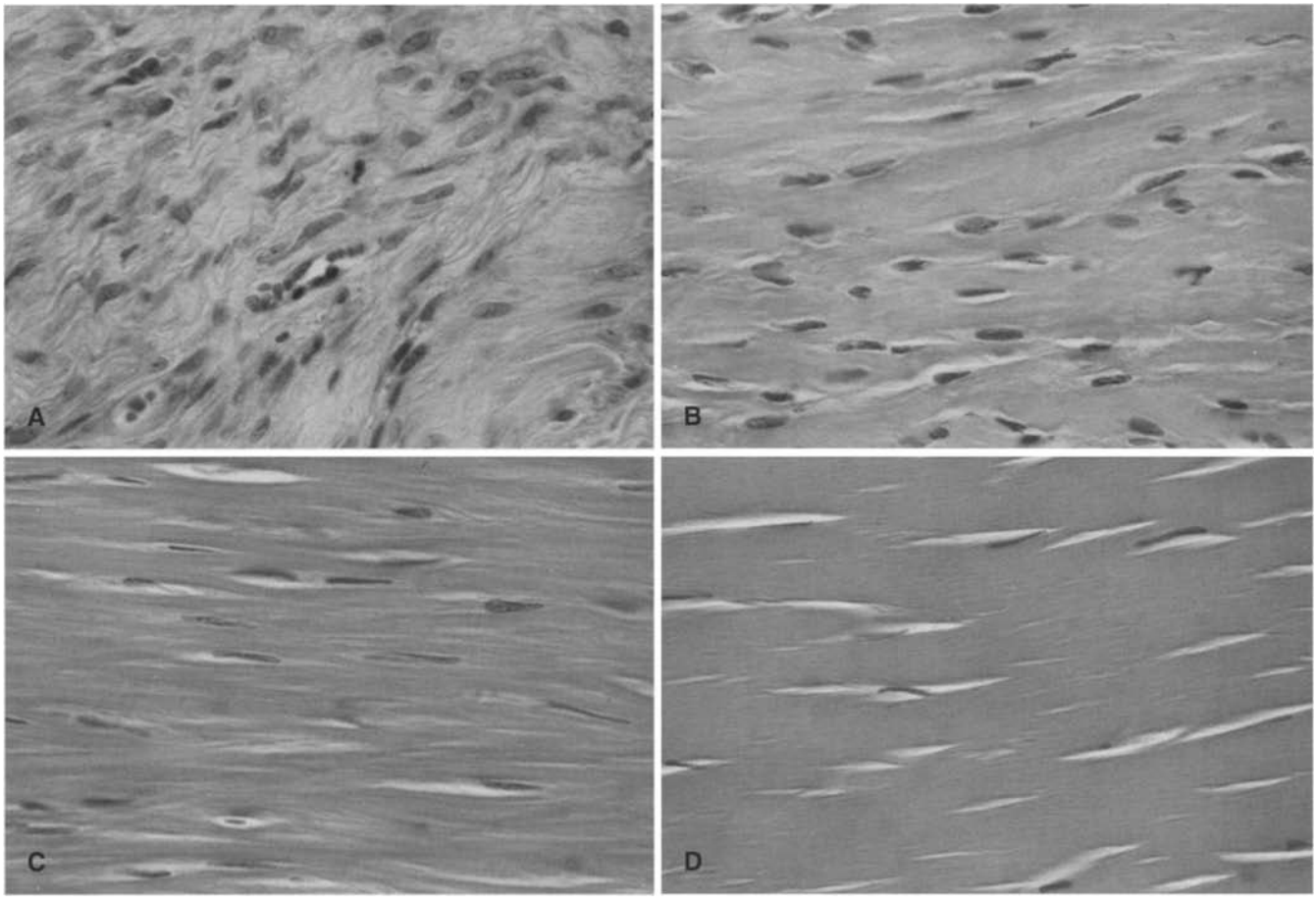


Fig. 5 Light microscopic appearance (H&E stain, × 160): **A** 1 month, **B** 3 months, **C** 6 months, **D** control. With time, the morphology of the nuclei changed from oval to club shape, and collagen fibers increased to run in a uniform direction

thickness of the regenerated tissue itself in the 6-month group averaged 0.9 ± 0.2 mm versus the control's 1.4 ± 0.2 mm, indicating that it was significantly thin. Hypertrophy of the regenerated tissue of the resected site was not observed.

Mechanical strength

The maximum load of the 3-month group in experiment 1 was 396.2 ± 112.2 N on the operated side vs the control's 401.5 ± 66.0 N, showing no apparent difference between

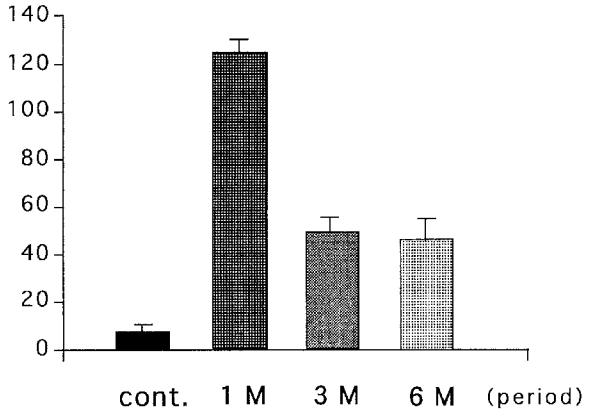


Fig. 6 Number of cells per visual field (× 160) showed a tendency to decrease with time, but even at 6 months was considerably larger than that of the control

the two. The maximum load of the 6-month group was 344.8 ± 104.3 N on the operated side vs the control's 313.6 ± 110.7 N, showing a slight increase in strength, but the difference between the two was not significant (Fig. 3). Upon examining the individual data, in one-half of the cases in both the 3-month and 6-month groups, the maximum load of the operated side including the regenerated tissue was found to be lower than that of the control with resection of the central one-third. Stiffness in the 3-month group was 123.7 ± 33.8 N/mm on the operated side vs the control's 155.8 ± 35.4 N/mm; thus, the former was significantly lower than the latter ($P < 0.05$). In the 6-month group, stiffness on the operated side was 125.4 ± 41.4 N/mm, being lower than the control's 142.8 ± 36.8 N/mm, but the difference was not statistically significant (Fig. 4).

The maximum load of the 3-month group in experiment 2 was 77.3 ± 31.1 N in the regenerated tissue vs 207.9 ± 101.0 N in the control, while in the 6-month group the maximum load was 78.7 ± 27.5 N in the regenerated tissue vs 303.4 ± 72.6 N in the control. The maximum load of the regenerated tissue in the 3-month group was 37.2% that of the control, and 25.9% that of the control in the 6-month group (Fig. 3). The stiffness in the 3-month group was 24.4 ± 10.0 N/mm in the regenerated tissue vs 101.8 ± 35.4 N/mm in the control. In the 6-month group stiffness in the regenerated tissue was 34.8 ± 19.7 N/mm vs 129.9 ± 19.7 N/mm in the control. The stiffness of the regenerated tissue in the 3-month group was 24% that of the control, and stiffness in the 6-month group was 26.8% that of the control (Fig. 4).

The failure mechanism in experiments 1 and 2 was mid-substance rupture of the patellar tendon in all cases. Avulsion from the bone did not occur.

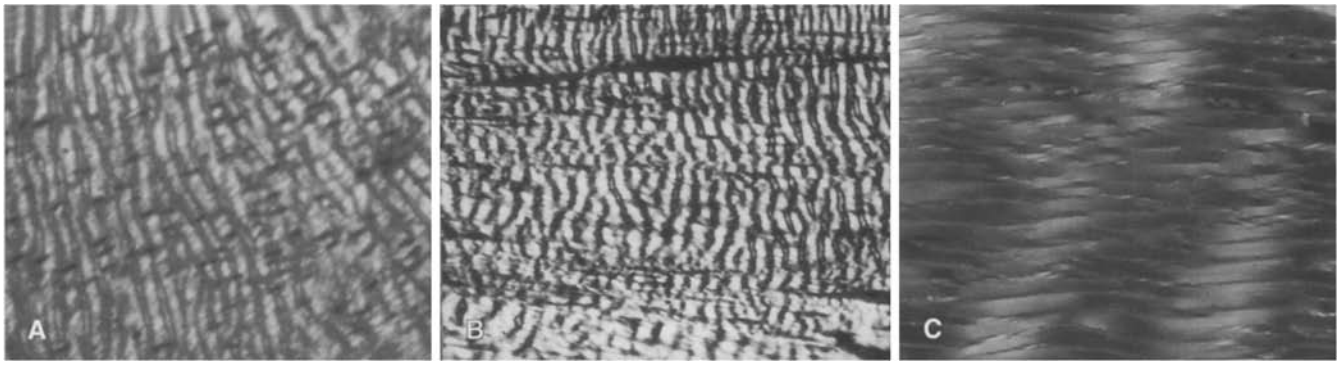
Histological findings

Collagen fibers could be observed at 1 month, but the amount was small, with the direction being irregular. Many fibroblast-like cells with oval-shaped nuclei could be seen. At 3 months, the number of collagen fibers had increased, and the direction had become almost consistent. The number of cells decreased, but the proportion of slender long nuclei increased. At 6 months, collagen fibers further increased, with club-shaped nuclei becoming predominant (Fig. 5). The number of cells per visual field at × 160 magnification was computed using the mean of three visual fields for each specimen. The mean of the control was 8.6, compared with 124.3 in the 1-month group, 50 in the 3-month group, and 46.6 in the 6-month group, showing a decline with time, but even at 6 months the number was high, being 5.4-fold that of the control (Fig. 6).

In the measurement of the crimp pattern of collagen fibers using a simple polarizing apparatus, the mean of the crimp period and crimp amplitude of the control were 117.5 and 13.8 μm, respectively, but those of the regenerated tissue were 13.3 and 2.7 μm in the 3-month group and those in the 6-month group were 13.6 and 2.6 μm, respectively. Even at 6 months, the crimp period and crimp amplitude were 11.5% and 18.5%, respectively, of the normal values (Figs. 7 and 8).

The cross-section of the collagen fibrils was examined under a transmission electron microscope. In the control, collagen fibrils with a diameter size extending up to 350 nm were comparatively evenly distributed, but in the regenerated tissue at 3 months and 6 months, most of the collagen fibrils had a small diameter up to 100 nm (Figs. 9 and 10).

Fig. 7 Polarized light appearance (× 80): **A** 3 months, **B** 6 months, **C** control. The crimp pattern of the 3-month and 6-month groups was evidently different from that of the control



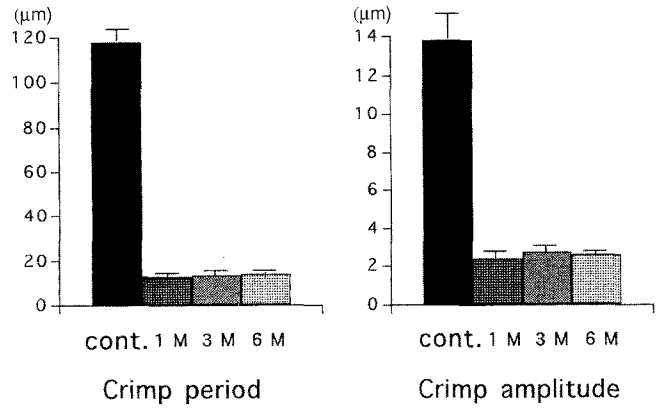


Fig. 8 Crimp pattern of the collagen fibers. Both the crimp period and crimp amplitude did not show much change up to 6 months, and when compared with the control, they were noticeably smaller

The area occupied by the collagen fibrils was evaluated by diameter size. In the control, most of the area was occupied by collagen fibrils with a large diameter, but in the 3-month and 6-month groups, the distribution was bimodal. In the latter, there was a tendency for the proportion occupied by collagen fibrils of large diameter to increase, but collagen fibrils of small diameter occupied more than 50% (Fig. 11). In addition, the total area occupied by collagen fibrils was computed. In the 3-month group, it was only 50.5% that of the control, while in the 6-month group it was 57.9%.

Supplement

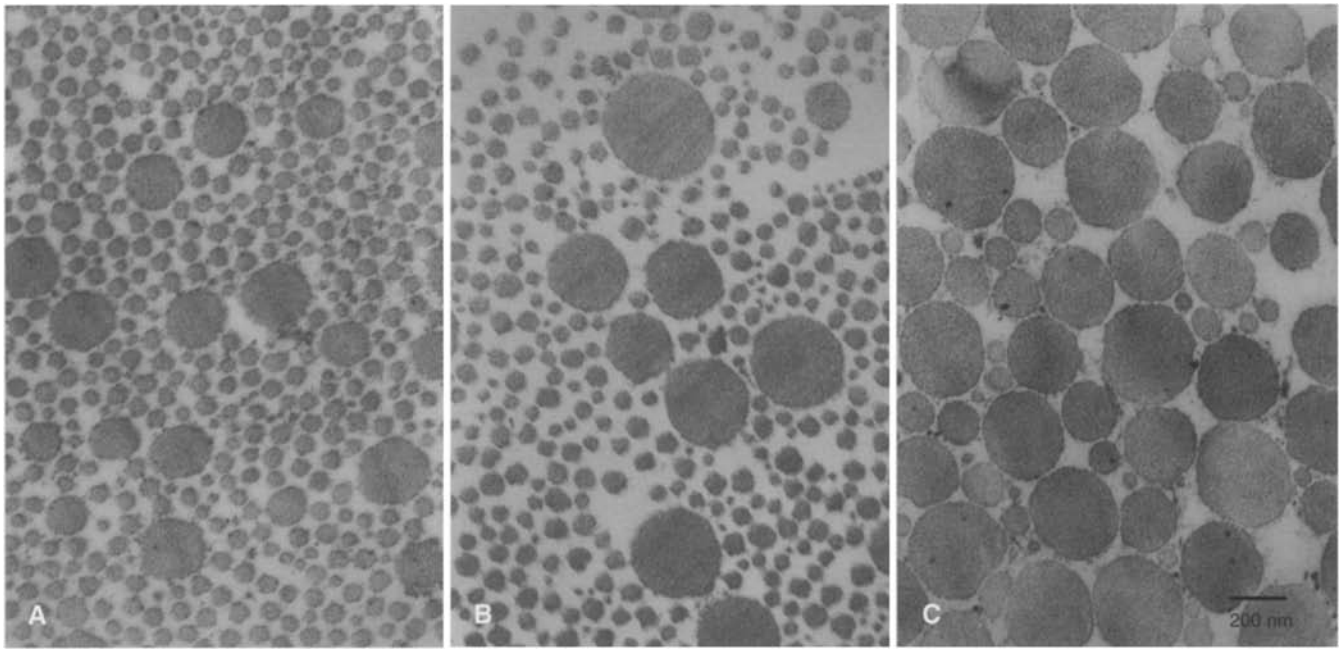
In specimens of experiment 1, the tissue of the medial side of the patellar tendon following removal of the central one-third was observed. In comparison with normal patellar tendon, the number of cells was large, and the collagen fibers were slender and irregular in 3 out of 8 cases in the 3-month group and in 4 out of 8 cases in the 6-month group, showing features close to those of regenerated tissue (Fig. 12).

Discussion

It was ascertained in the present study that though the defect located in the central patellar tendon was filled with regenerated tissue, the mechanical strength of the regenerated tissue even at 6 months was remarkably weak and the detailed structure of the collagen fibers was qualitatively immature.

Concerning the mechanical strength of the patellar tendon after removal of its central one-third, Burks et al. in a detailed report of dogs observed that the maximum load at 3 months and 6 months was 70% and 60%, respectively, of that of the patellar tendon on the normal side (control). As the cross-section of the patellar tendon appeared to be spindle-shaped and removal of the central one-third accounts for resection of about 40%, the results of Burks et al. suggest that the regenerated tissue has hardly any strength, but no reference was made to any direct measurement. Furthermore, the usual failure mechanism was avulsion from the bone. Such data do not reflect the strength of the tendon itself. Therefore, we prepared models of two groups. In experiment 1, the entire patellar tendon including the regenerated tissue at the defect site (operated side) was compared with the bilateral two-thirds of

Fig. 9 Transmission electron microscopic appearance of the cross-section of collagen fibrils: **A** 3 months, **B** 6 months, **C** control. At 3 months and 6 months, the number of collagen fibrils with small diameter was apparently larger than that of the control



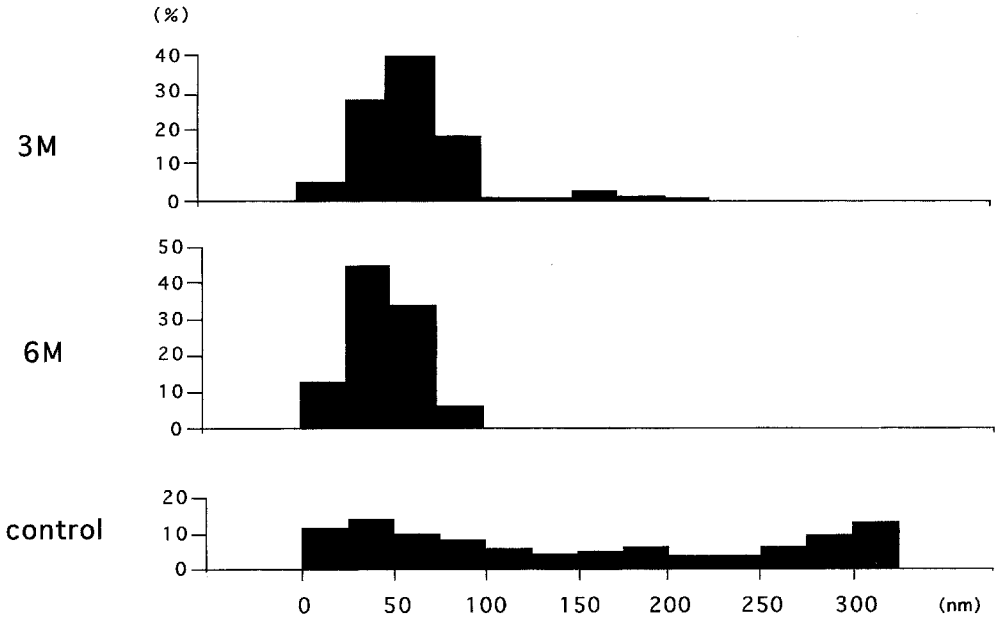


Fig. 10 Distribution of collagen fibrils by diameter size. In the control, collagen fibrils of large and small diameter were comparatively evenly distributed, but at 3 months and 6 months, most of the collagen fibrils were of small diameter

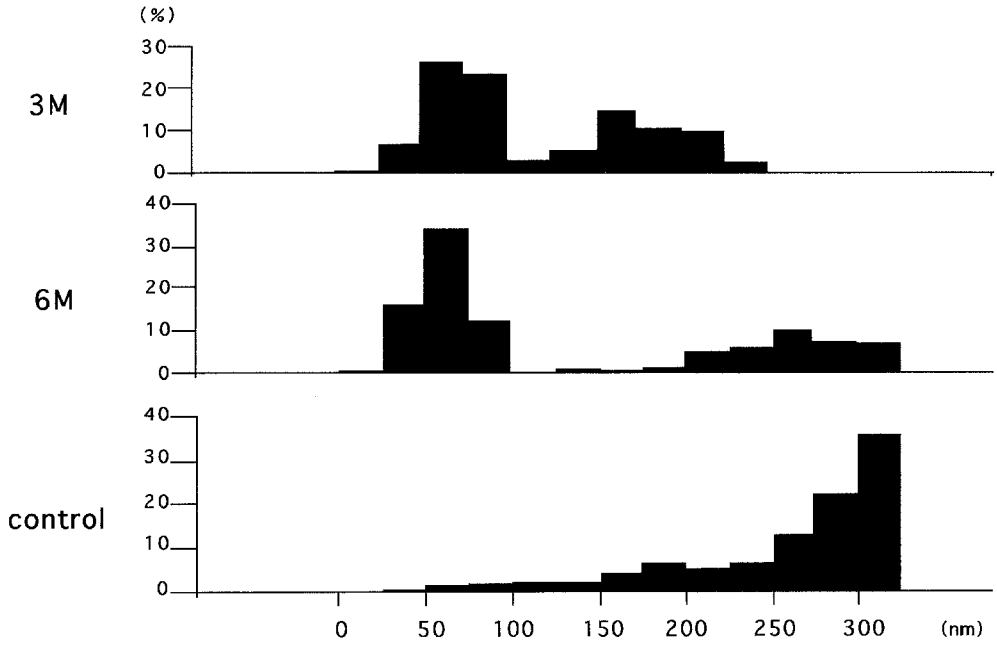


Fig. 11 Percentage of area occupied by collagen fibrils. At 3 months, collagen fibrils of intermediate diameter could be observed, but at 6 months, the proportion occupied by collagen fibrils of large diameter had increased

the patellar tendon after removal of its central one-third at the time of killing (control). In experiment 2, only the regenerated tissue at the central defect site (operated side) was compared with only the central one-third of the patellar tendon with bilateral resection of two-thirds at the time of killing (control). From this, the strength of the regenerated tissue was evaluated. Furthermore, to avoid avulsion from the bone, one- to two-thirds of the tendon was resected so that the strength of the tendon would be relatively lower than the strength of the bone, and in addition, the bone was fixed by covering with resin. In experiment 1, no difference was observed in the mean values, indicating that the regenerated tissue itself does not contribute to strength. However, on examination of the individual data, in about one-half of the cases, the strength of the operated side including the regenerated tissue was weak, suggesting weakening of the remaining patellar tendon on the medial and lateral sides. Stiffness on the operated side was 79.4% that of the control in the 3-month group, which is significantly reduced. Though no significant difference was observed in the 6-month group, the mean value of the operated side was lower than that of the control. As alluded to earlier in experiment 1, as the strength of the remaining patellar tendon on the medial and lateral sides had changed, it was not possible to ascertain the strength of the regenerated tissue itself. In experiment 2, the directly measured maximum load of the regenerated tissue was 77.3 ± 31.3 N in the 3-month group and 78.7 ± 27.5 N in the 6-month group, showing no difference between the two. Even in the 6-month group, it was 25.9% of the

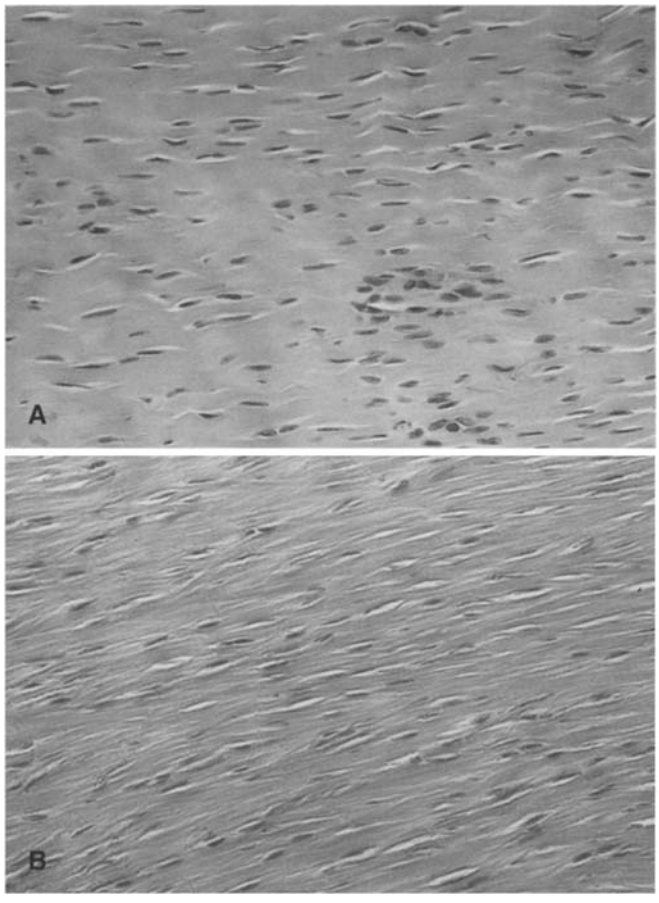


Fig. 12 Medial portion of the residual patellar tendon after removal of the central one-third (H&E stain, × 80): **A** 3 months, **B** 6 months. In both the 3-month and 6-month groups, the morphology of most of the nuclei was oval or slender, and the collagen fibers were slender and irregular

central one-third of the patellar tendon used as a control. Furthermore, stiffness of the 6-month group was only 26.8% of that of the control. Thus, there was no increase in maximum load during the period from 3 months to 6 months, the maximum load of the regenerated tissue at 6 months was only about one-fourth that of normal tissue, and there were cases of reduction in strength of the residual patellar tendon on the medial and lateral sides. As possible causes of this reduction of strength of the patellar tendon on the medial and lateral sides following resection, direct operative invasion and disturbance of normal blood flow may be considered. However, as many cases of elongation of the patellar tendon on the operated side were noted, as a histological appearance resembling that of regenerated tissue was often observed on the medial side of the patellar tendon (as described in the Supplement), and as the histological appearance of rupture of normal tendon tissue with the presence of regenerated tissue at such a site was seen, it is speculated that it was a result of tissue damage which developed due to the inability to sustain an excessive load.

As for the postoperative thickness of the patellar tendon, there are many reports such as those by Berg and Coupens et al. that the strength of the removed tissue is compensated by thickening. In the present experiment, the regenerated tissue of the central resected site was significantly thinner than the patellar tendon. However, proliferation of scar-like connective tissue was observed on the anterior surface of the patellar tendon, and when this is included, there is thickening of the patellar tendon, which could be interpreted as hypertrophy on MRI. As the mechanical test was conducted after resecting this connective tissue in the present study, the possibility that the proliferated connective tissue bears some of the load and that the extensor retinaculum has thickened and compensated for the weakened patellar tendon cannot be denied. However, the proliferated tissue on the surface of the patellar tendon is a soft connective tissue which can be readily distinguished from the patellar tendon tissue; it is rather unlikely that this tissue bears a large load.

In the light microscopic observation made at 6 months, the morphology and arrangement of the cells and collagen fibers of the regenerated tissue at the resected site approached those of normal tissue with time, and stress was applied to the regenerated tissue. However, in studying the crimp pattern which characterizes tendon and ligament, it was found even at 6 months that the crimp period and crimp amplitude were apparently small, being 11.5% and 18.5%, respectively, of the normal tissue, suggesting that the buffer mechanism of collagen fibers, which are sinuous in structure, is depressed. Furthermore, though few in number, collagen fibrils of intermediate diameter were observed at 3 months, and those of large diameter at 6 months, but even at 6 months the diameter of most of the collagen fibrils remained small (less than 100 nm). This is evidently different from mature patellar tendon tissue. The total area occupied by collagen fibrils in the cross-sectional area was 57.9% that of the control at 6 months, which is a finding supporting the reduction of strength.

In a clinical case in which a part of the regenerated tissue was obtained 1 year postoperatively using the central one-third of the patellar tendon, the light microscopic findings and the crimp pattern were almost identical to the findings of the tissue for up to 6 months in the present study. These details support our experimental results that a long period is required for the structure and function of collagen fibers of the regenerated tissue to be restored to normal.

The rabbit, a knee-bending animal, was used in the present study, and as postoperative therapy, immobilization and rest were not provided. It is therefore presumed that the patellar tendon bore a large load from an early stage. Though the results of the present study cannot be directly applied to humans, in the case of active athletes who demand a quick recovery, postoperative therapy should be provided with consideration given to the possibility that the decrease in mechanical strength can lead to patellar tendon damage.

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