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In Vivo Effects of Partial Electrothermal Shrinkage on Mechanical Properties of the Anterior Cruciate Ligament in Rabbits

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Footnotes

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Abstract

Background: No studies have been conducted to clarify an *in vivo* remodeling of the radiofrequency-treated lesion of the anterior cruciate ligament. The purpose was to determine *in vivo* effects of radiofrequency shrinkage on mechanical properties of the anterior cruciate ligament.

***Methods*:** Thirty skeletally mature rabbits were used. In each group, radiofrequency energy set at non-ablative levels was applied to the posterolateral bundle of the anterior cruciate ligament with a bipolar radiofrequency generator. All animals were sacrificed at 0, 6, and 12 weeks after surgery, respectively. In each group, 7 and 3 out of the 10 specimens were used for biomechanical and histological evaluations.

Findings: After shrinkage treatment, the anterior-posterior translation of the knee and the length of the posterolateral bundle were significantly reduced immediately after surgery, but that this effect disappeared at 6 weeks. The tensile strength and the tangent modulus of the treated bundle were significantly lower than that of the normal control bundle at each period. In addition, the tensile strength and the tangent modulus measured at 12 weeks were significantly lower than that at 0 week. Histological examination showed granulation–like tissues with numerous plump fibroblasts and inflammatory cells were dominantly found in the midsubstance of the posterolateral bundle at 12 weeks.

Interpretations: This result suggested that the anterior cruciate ligament tissue shortened with the radiofrequency treatment is elongated gradually over time. The mechanical properties of the posterolateral bundle of the anterior cruciate ligament reduced by the radiofrequency shrinkage are not restored *in vivo*, but significantly deteriorated with time.

Key words: Anterior Cruciate Ligament; Biomechanics; Radiofrequency; Shrinkage; In vivo study

1. Introduction

Radiofrequency (RF) energy has been clinically applied to shrinkage of capsular tissues in the glenohumeral joint to restore joint stability (Abelow, 1997; Fanton, 1998; Levy et al., 2001), based on *in vivo* and *in vitro* basic studies (Obrzut et al., 1998; Hecht et al. 1998, 1999). Recently, several clinical reports have dealt with an application of the RF energy to the anterior cruciate ligament (ACL) injuries (Carter et al. 2002; Farng et al., 2005; Halbrecht, 2005; Indelli et al., 2003; Perry and Higgins, 2000; Sekiya et al., 2000; Spahn and Schindler, 2002; Thabit, 1998). Some *in vitro* studies, however, have been conducted to evaluate effects of RF energy on biomechanical properties of the normal or injured ACL (Dodds et al., 2004; Kondo et al. 2005; Ma et al., 2005; Ozenci and Panjabi, 2003, 2005). Our previous *in vitro* study (Kondo et al. 2005) demonstrated that the RF energy drastically reduces structural properties of the ACL immediately after surgery, dependent of its magnitude, while the RF energy significantly reduces the length of the ACL. Recently, a few *in vivo* studies have shown that RF energy significantly deteriorates structural properties of the whole ACL (Lopes and Markel, 2003; Scheffler et al., 2005). In the common shrinkage treatment for the ACL, however, RF energy is applied to only the anterior aspect of the ACL so that there are treated and intact portions in a cross-section of the ACL. Therefore, the previous *in vivo* studies did not determine the mechanical properties of the RF-treated lesion in the ACL, distinguishing from those of the untreated ligament tissue.

In the clinical field, many orthopaedic surgeons have expected that the ACL properties reduced by the RF shrinkage treatment may be gradually restored, when some ligament tissues remain intact around the RF-treated lesion (Indelli et al., 2003;

Thabit, 1998). No studies, however, have been conducted to clarify an *in vivo* remodeling course of the RF-treated lesion of the ACL, distinguishing from the untreated portion. Based on our *in vitro* study (Kondo et al. 2005), we have hypothesized that the mechanical properties of the ACL reduced by the RF shrinkage are not restored at 12 weeks after treatment, even when sufficient volume of ligament tissues remains intact around the treated lesion. The purpose of this *in vivo* study is to test this hypothesis.

2. Methods

2.1. Study Design

A total of thirty skeletally mature female Japanese White rabbits weighing 3.5 (standard deviation, 0.2) kg were used in this study. Animal experiments were carried out in the Institute of Animal Experimentation, Hokkaido University School of Medicine, under the Rules and Regulations of the Animal Care and Use Committee. In each animal, the right ACL was treated using the following quantitative technique. Surgery was performed under anesthesia induced by an intravenous injection of pentobarbital (25 mg/kg). In each animal, the right knee was positioned at 90 degrees of knee flexion by stabilizing the lower leg attached to an operating table. In a sterile fashion, the ACL was exposed through the medial parapatellar approach. After the anteromedial (AM) and posterolateral (PL) bundles of the ACL were identified, and were then separated by a blunt probe between the two bundles (Woo et al., 1992). A loose circumferential ligation was made with 5-0 black nylon suture at the proximal and distal portion of the PL bundle to give a landmark for postoperative evaluations (Fig. 1). Then, RF energy was applied to the midsubstance of the PL bundle with a commercially available medical

device composed of a bipolar RF generator (Multi Electrode System 2000; Arthrocare, Sunnyvale, CA, USA) and a probe (Arthrowand A1630-01; CAPSX, Arthrocare, Sunnyvale, CA, USA) (Fig. 1). This probe contains 12 electrodes in an end having a diameter of 3.0 mm. RF energy was set at a non-ablative level (Level 1; 28 Watts). This level was chosen, because it is recommended for clinical usage. The RF treatment was performed for 5 seconds, at each stroke, simulating clinical arthroscopic surgery using the continuous small stroke technique (approximately 1 to 2 mm/seconds) (Hayashi et al. 1995). Briefly, a surgeon (E.K.) approached the midsubstance of the PL bundle with the probe from only the anterior direction. Only the PL bundle was treated in physiological saline solution at 90 degrees of knee flexion, because our pilot study showed that, when the AM bundle was treated in the same manner, the AM bundle was completely torn at 6 weeks. The incised joint capsule and the skin wound were closed in layers with 3-0 nylon sutures, and an antiseptic spray dressing was applied. No immobilization was applied after surgery. The animals were allowed unrestricted activities in their cages (52 cm in width, 35 cm in height, and 33 cm in depth). Then, all the animals were randomly divided into 3 groups, Groups S0, S6, and S12, with 10 rabbits in each group. In these groups, all animals were sacrificed at 0, 6, and 12 weeks after surgery, respectively. In each group, 7 out of the 10 rabbits were used for biomechanical evaluation, and the remaining 3 were used for histological observation with light microscopy. Nine knees (Control group) randomly harvested from all left knees were used to obtain normal control data.

2.2. Measurement of the anterior-posterior translation of the knee

At the time of sacrifice, the lower extremities of each animal were then

117 disarticulated at the hip joint. Each specimen was stored at –80 degrees Celsius until the
118 time of testing. Prior to mechanical testing, each knee was thawed overnight at 4
119 degrees Celsius. The anterior-posterior translation of the knee was determined as
120 follows. The femur–knee–tibia complex (45 mm–long femur and 60 mm–long tibia)
121 was removed from the hindlimb. All the surrounding muscles were carefully dissected.
122 Care was taken to avoid injuring the joint capsule and the ligament tissues. The femur
123 and the tibia were separately cast in cylindrical aluminum tubes (25–mm diameter and
124 30–mm length) using polymethylmethacrylate resin. The specimen was mounted onto a
125 specially designed testing device having 3–degrees of freedom, which was attached to a
126 tensile tester (RTC–1210, Orientec, Oakabe, Japan) (Kondo et al., 2003). Four cycles of
127 anterior-posterior shear loads of 10 N were applied to the knee specimen at 30, 60, and
128 90 degrees of knee flexion, respectively. A cross–head speed was set at 5 mm/min. The
129 specimen was kept moistened throughout the test period with a physiologic saline
130 solution spay. Load-elongation curves were drawn with an X–Y recorder (Model 3023,
131 Yokogawa, Tokyo, Japan). The maximum anterior-posterior translation in the
132 load-elongation curve was defined as the anterior-posterior translation of the knee at
133 each angle of knee flexion.
134
135 ***2.3. Measurement of the tissue dimension***
136 Then, the joint capsule and all ligaments except for the ACL were carefully
137 dissected in each specimen. Synovium-like tissues that enveloped the ACL were
138 removed for tensile testing. The PL bundle sutured with 5-0 nylon was easily identified.
139 The AM bundle of the ACL was resected using a stainless-steel razor blade to determine
140 the mechanical properties of the RF-treated bundle distinguished from those of the

untreated bundle. The femur was clamped with the alligator jaw attached to a steel stand, and the tibia was suspended from the femur with the PL bundle of the ACL. A weight was attached to the distal end of the tibia so that a 0.5-N load was applied to the PL bundle of the ACL. The femur was inclined so that the knee was flexed at 45 degrees. The length of the treated bundle of the ACL was measured with a vernier caliper (Mitutoyo, Kanagawa, Japan) at the anterior, posterior, medial, and lateral aspects, respectively. The mean of the four length values was defined as the length of the treated bundle of the ACL.

The cross–sectional area of the treated PL bundle of the ACL was measured under the same condition as the optical non-contact method using a CCD camera (WV–BD400, Panasonic, Osaka, Japan) and a video dimension analyzer (HTV–C1170, Hamamatsu Photonics, Tokyo, Japan), which was reported by Yamamoto et al. (1999) Briefly, the medial femoral condyle and a portion of the lateral femoral condyle distal to the ACL insertion were resected for visualization with the video dimension analyzer. The femur was attached to the stepping motor and a constant tensile load of 0.5 N was applied to the ACL by suspending a weight to the tibia. The femur was rotated with the stepping motor at 5–degrees angular increments through 360 degrees, and the corresponding profile width of the ACL was recorded with the video dimension analyzer. The cross–sectional shape of the ACL was reconstructed using a computer algorithm. The measurement was done at the middle of the ACL in order to quantify a part of gross observation on the thickness of the ACL.

2.4. Tensile testing

Then, the prepared femur–PL bundle–tibia complex specimen was mounted

onto a conventional tensile tester (PTM-250W; Orientec, Tokyo, Japan) (Fig. 2). The tibia was flexed at 45 degrees against the femur. The knee was rotated approximately 90 degrees toward the internal direction to remove the normal distortion of the ACL (Woo et al., 1992), although the loads were not completely applied to all portions of the bundle during tensile testing. Two parallel lines were drawn transversely on the surface using nigrosine stains as gauge-length markers for strain measurement. The distance between the 2 lines was approximately 7 mm. Before the tensile test, the specimen was preconditioned with a static preload of 0.5 N for 5 minutes, followed by 10 cycles of loading and unloading (3% strain) with a crosshead speed of 5 mm/min. Then, each specimen was stretched to failure at a crosshead speed of 20 mm/min. Elongation in the ligament substance was determined with a video dimension analyzer using the gauge-length markers. We determined the tensile strength and the tangent modulus of the PL bundle based on the data of cross-sectional area of the PL bundle, a load cell, and a video dimension analyzer (Fig. 2).

2.5. Histological observations

In each limb intended for histological observation, the femur–PL bundle–tibia complex was resected and fixed in a buffered 10 % formalin solution, decalcified, and cast in paraffin blocks. For each block, we set a microtome so that the midsubstance was sectioned parallel to the longitudinal axis. The central portion of the whole specimen in the sagittal plane was marked on the block surface. The half of the specimen was resected off until the central sagittal plane could be observed. Then, five 5-μm continuous sections were obtained, and they were stained with hematoxylin and eosin for histological observations. Two of the coauthors (KY, HT) observed the

histologic evaluation, independently, under a blinded manner. Cellularity, shape of the nucleus of cells, and collagen striations in the ligament substance were observed with light microscopy.

2.6. Statistical Analyses

All data were shown as the mean with the standard deviation value. Concerning each parameter, the one–way analysis of variance (ANOVA) was performed among the groups. When a significant effect was obtained, a post–hoc test with the Fisher's protected least significant difference test was made for multiple comparisons. A commercially available software program (Stat View; SAS Institute, Cary, NC, USA) was used for statistical calculation. The significance level was set at $p = 0.05$.

3. Results

3.1. Anterior-posterior translation of the knee

Concerning the anterior-posterior translation of the knee, the ANOVA showed a significant difference among the groups at each angle of knee flexion ($p<0.0265$). The post–hoc test demonstrated that, at 60 degrees of knee flexion, Group S0 was significantly shorter than Groups S6 ($p<0.0001$), S12 ($p<0.0001$), and the control group ($p<0.0001$), while there were no significant differences among Groups S6, S12, and the control group (Table 1).

3.2. Gross observation of inside the knee joint

The PL bundle of the ACL was obviously shortened immediately after RF treatment. The treated ACL appeared to be more translucent in Group S0 (Fig. 3–A),

compared to normal control. In the treated groups, the PL bundle of the ACL was enveloped by thin synovium-like tissues in Groups S6 (Fig. 3–B) and S12 (Fig. 3–C). In these two groups, the treated portions could be distinguished from the other portions in the midsubstance.

3.3. Tissue dimension of the posterolateral bundle of the ACL

After the RF treatment, the ANOVA demonstrated a significant difference (p=0.0098) in the PL bundle length among the 4 groups (Table 1). The post-hoc test showed that the length of Groups S0 was significantly shorter than that of the normal control (p=0.0037) and Group S12 (p=0.0038). There were no significant differences between the normal control, Groups S6, and S12 (Table 1).

Regarding the cross–sectional area of the PL bundle, the ANOVA demonstrated a significant difference (p=0.0263) among the groups. The post-hoc test showed that Groups S0 were significantly greater than Groups S6 (p=0.0065), and S12 (p=0.0237), respectively. There were no significant differences between the Groups S6, and S12 (Table 1).

3.4. Mechanical properties of the posterolateral bundle of the ACL

In tensile testing, failure modes showed that five specimens were torn in the midsubstance of the bundle in the control group, while all specimens failed at the midsubstance in Groups S0, S6, and S12. Only the specimens torn in the midsubstance of the bundle were used to determine the mechanical properties of the PL bundle. The stress-strain curves indicated that obvious differences were observed between the control group and the other 3 treated groups (Fig. 4).

In tensile testing, the ANOVA demonstrated a significant difference in the tensile strength among the groups (p<0.0001). The post-hoc test showed that Groups S0, S6, and S12 were significantly lower than the control group, respectively (p<0.0001). In addition, Group S12 was significantly lower than Group S0 (p=0.0159) (Table 2). Concerning the tangent modulus, the ANOVA demonstrated a significant difference among the groups (p<0.0001). Groups S0, S6 and S12 were significantly lower than the control group, respectively (p<0.0001). Group S12 was significantly lower than Group S0 (p=0.0032) (Table 2). Regarding the strain at failure, the ANOVA revealed no significant differences among the 4 groups (Table 2).

3.5. Histology

In the control group, the normal ligament was covered by a thin synovial membrane. The midsubstance consisted of closely packed collagen fibers, which were aligned longitudinally with a periodic crimp pattern. Fibroblasts were sparsely scattered between the collagen fibers (Fig. 5–A). Histological examination performed immediately after the treatment showed diffuse collagenous denaturation and pyknotic nuclear changes in fibroblasts in the RF treated portion. The crimp patterns were not present in the treated area (Fig. 5-B). In Groups S6 and S12, granulation–like tissues with numerous plump fibroblasts and inflammatory cells were dominantly found in the midsubstance of the PL bundle, where collagen fibers were loosely woven without the crimp pattern (Fig. 5–C).

4. Discussion

First, this study has been clarified an *in vivo* effects of the RF shrinkage on mechanical properties of the ACL. After shrinkage treatment, the anterior-posterior translation of the knee and treated bundle length were significantly reduced immediately after surgery, but that this effect disappeared at 6 weeks. This result suggested that treated bundle tissue shortened with the RF shrinkage treatment is elongated gradually over time. Concerning the mechanical properties, the tensile strength and the tangent modulus of the treated bundle were significantly lower than that of the normal control bundle at each period. In addition, the tensile strength and the tangent modulus measured at 12 weeks were significantly lower than that at 0 week. This study clearly demonstrated that, even when sufficient volume of ligament tissues remains intact around the treated portion, the mechanical properties of the ACL reduced by the RF shrinkage are not restored *in vivo*, but significantly deteriorated with time. This result indicated that the intact ligament tissue around the treated portion does not protect the RF-treated ACL tissue from the material deterioration.

The alternation that occurred in the mechanical properties of the RF treated bundle tissue was well illustrated by the histological findings of this tissue. Immediately after treatment, the tissue damage was characterized by the fused and homogenized appearance of collagen tissues accompanied with the nuclear pyknosis of fibroblasts. At 6 and 12 weeks, granulation–like tissues with numerous plump fibroblasts were predominantly found in the midsubstance of the ACL, where collagen fibers were loosely woven without the crimp pattern. These histological changes observed in this study are similar to those previously reported in the literature that dealt with the effect of the thermal energy not only on the capsular tissues of the joint (Hecht et al. 1998, 1999; Lu et al. 2000) but also on the tendon and ligament tissues (Schaefer et al. 1997;

284 Vangsness et al. 1997). It is considered that the alternation of collagen fiber structure
285 resulted in the deterioration of the mechanical properties of the ACL.

286 In this study, the application of RF energy to the PL bundle of the ACL does
287 not affect the joint stability after 12 weeks. The ACL consists of 2 distinct bundles, the
288 AM and PL bundles, and these bundles contribute synergistically to the stability of the
289 knee. Gabriel et al. (2004) investigated the in situ forces of the 2 functional bundles of
290 the ACL and showed higher in situ forces in the PL bundle close to knee extension when
291 compared with the AM bundle. Recently, Zantop et al. (2007) reported that isolated
292 transsection of the PL bundle increased anterior tibial translation at 30 degrees of knee
293 flexion significantly compared with the intact knee. Concerning the PL bundle length,
294 there was no significant difference between week 12 and the control. In addition, at the
295 measurement of the joint stability, the anterior-posterior shear loads chosen were 10 N,
296 which was a low load of the failure stress of the normal ACL in rabbits. Therefore,
297 although the tangent modulus and the cross-sectional area of the PL bundle were
298 decreased from week 0 to week 12, the joint stability at 30 degrees, 60 degrees, and 90
299 degrees of knee flexion after 12 weeks was not less than the control.

300 Recently, several *in vitro* basic studies have been conducted to evaluate effects
301 of RF energy on biomechanical properties of the normal or injured ACL (Dodds et al.,
302 2004; Kondo et al. 2005; Ma et al., 2005; Ozenci and Panjabi, 2003, 2005). It was
303 shown that deleterious effects of subfailure injury were restored by RF treatment in an
304 *in vitro* rabbit ACL study (Dodds et al., 2004). However, Ozenci and Panjabi (2005)
305 examined the effects of cyclic loading on a thermally treated injured ACL *in vitro*. They
306 reported that relaxation forces first increased after the RF treatment, and then decreased
307 after cyclic loading. Although their studies were an *in vitro* study, our findings were

similar: shrinkage after RF treatment and, increased laxity after surgery. In this study, the anterior-posterior translation of the knee was significantly reduced immediately after surgery, but that this effect disappeared at 6 weeks.

Concerning the *in vivo* effects of RF treatment, Hecht et al. (1999) reported that the stiffness of the joint capsular tissue that had been reduced by the RF treatment returned to a normal value by 6 weeks. However, Lopez and Markel (2003) demonstrated that the canine ACLs treated with monopolar RF energy were torn approximately 55 days after treatment. In addition, Scheffler et al. (2005) reported the effect of RF shrinkage on the structural properties of the elongated ACL in a sheep model. They investigated that the initial reduction of knee laxity after RF treatment could not be maintained at 24 weeks. A significant reduction in ultimate load was found at 24 weeks in the RF-treated group compared with the untreated group. Schaefer et al. (2005), who studied the *in vivo* response to laser thermal treatment in the rabbit patellar tendon, reported that the treated tendons were stretched out within 4 weeks after treatment. Wallace et al. (2002) stated that the thermal shrinkage to the relaxed medial collateral ligament of the rabbit increased the creep deformation and the possibility of failure by low physiologic stresses. In addition, it has been well known that healing capacities of the ACL is lower that that of the extra-articular ligament and capsular tissues (Amiel et al. 1990; Arnoczky et al. 1979) Therefore, we have to be pessimistic concerning the early restoration of the mechanical properties of the ACL reduced by the RF treatment.

As to clinical relevance of this study, recently, a few clinical studies dealing with the electrothermal shrinkage for the elongated ACL have been reported. Thabit (1996) treated 25 patients with the relaxed native ACL or the reconstructed ACL that

were lax but in continuity, using a RF device. They described that the KT-1000 arthrometer value was within 2 mm of that for the normal knee in 23 patients at the time of 1.5 years or more after surgery. Spahn and Schindler (2002) also reported favorable 9 month follow-up results after RF treatment for 14 patients with secondary instability after ACL reconstruction. Indelli et al. (2003) studied 28 patients with symptomatic ACL laxity but with continuity of the ligament. After, monopolar RF treatement, 27 of 28 patients rated their knees as normal or nearly normal at minimum follow-up of 2-years and had KT-1000 side-to-side differences of less than or equal to 3 mm. On the other hand, Carter et al. (2002) applied the electrothermal treatment for 18 patients who had continuity of the ACL but had symptomatic laxity. However, a poor result was obtained in 11 patients within several months after surgery. In addition, Perry and Higgins (2000) reported a case of spontaneous, simultaneous rupture of both the ACL and PCL 3 months after the electrothermal treatment under minimal physiologic load. Sekiya et al. (2000) reported a case of autodigestion of the ACL after the electrothermal treatment. Recently, Halbrecht (2005) reported the long-term failure of thermal shrinkage for laxity of the ACL. 19 patients with partial tears of the ACL or stretched ACL grafts underwent thermal shrinkage treatment using monopolar RF device. At 5-year follow-up, 11 of 13 patients had gone on to complete failure. They stated that thermal shrinkage provides short-term benefit in the treatment of ACL laxity but leads to catastrophic failure in the majority of patients at long term follow-up. Our study may explain one of the causes of the poor results in these clinical reports. However, we have to recognize that long-term follow-up studies have not been conducted with a sufficient number of patients to clarify the clinical utility of the electrothermal treatment for the ACL.

There are some limitations of this study. The first limitation of this study is that we used the rabbit ACL. However, we must recognize that the absolute values shown in this study are not completely equivalent to those obtained from the human ACL. Secondly, this study dealt with the uninjured ligament to obtain the most fundamental effect on the ACL. Therefore, we could not precisely refer to the effect to the injured ACL. However, the mechanical properties of the injured ACL have already been deteriorated. Therefore, we can speculate that RF treatment for the injured ACL may additionally reduce the deteriorated mechanical properties. This may increase the rate of ACL rupture. Thirdly, limitation was that we could not quantify histological findings. Fourthly, the system was designed to deliver therapeutic temperatures to a depth of 0.35 mm thickness used at a voltage setting of the Level 1. Concerning the tissue temperature at the time of the treatment with this system, Foster and Elman (1998) described that, within 2-3 seconds using this setting of 1, the temperature at the tissue surface became approximately 75 degrees, while the temperature of the tissue at 0.35 mm thickness became approximately 62 degrees.

Finally, this study clearly demonstrated that the mechanical properties of the RF-treated lesion in the ACL, distinguishing from those of the untreated ligament tissue, were drastically reduced after shrinkage treatment. We conclude that non-ablative RF energy induces significant deterioration of the mechanical properties of the ACL, although the electrothermal treatment can effectively shorten the ACL immediately after surgery. Furthermore, the mechanical properties of the ACL tissue reduced by the RF shrinkage are not restored *in vivo*, but significantly deteriorated after 12 weeks. Therefore, this study warned against too optimistic application of electrothermal shrinkage to the ACL as a clinical treatment. At the present time, thermal shrinkage is

only a clinical option to shorten the elongated ligament tissues. We should take the biomechanical reduction of the RF-treated tissue into consideration when determining postoperative rehabilitation of the treated tissue.

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Figure legend

Figure 1.

Operative photograph before the radiofrequency treatment.

Figure 2.

Apparatus for tensile testing

Figure 3–A, B, C.

Operative photograph at 0 (A), 6 (B), and 12 weeks (C) after the radiofrequency treatment. A circumferential ligation was made with 5-0 black nylon suture at the middle portion of the AM bundle for resection of the AM bundle.

Figure 4.

Stress-strain curves for the femur–PL bundle–tibia complexes. Each error bar represents the standard deviation.

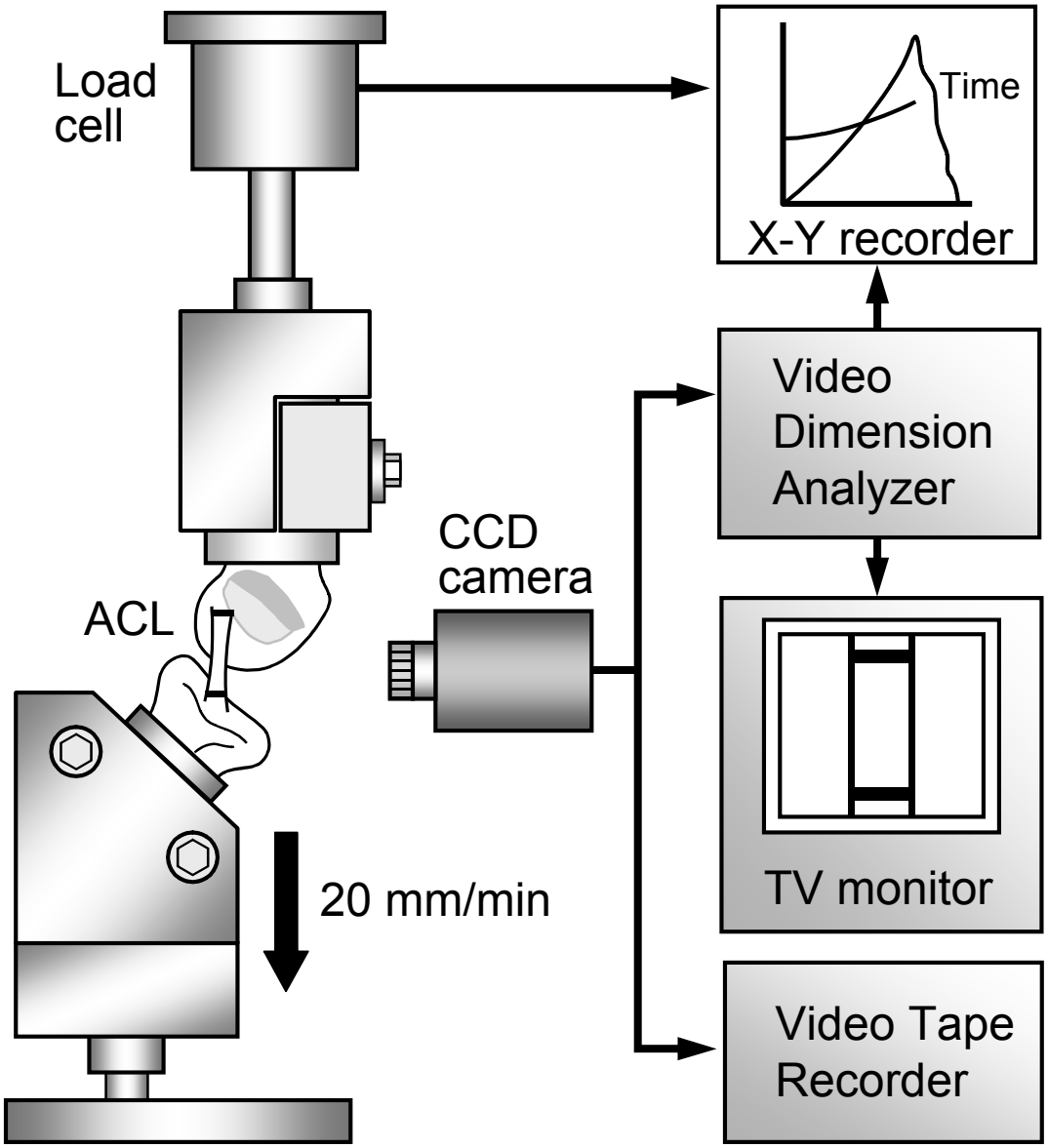
Figure 5-A,B,C.

Histological findings in the midsubstance of the posterolateral bundle of the ACL with light microscopy. **A:** Histology of the midsubstance of the normal ACL with light microscopy. **B:** Histological examination performed immediately after the treatment showed diffuse collagenous denaturation and pyknotic nuclear changes in fibroblasts in the RF treated portion. The crimp patterns were not present in the treated area. **C:** In Group S12, granulation–like tissues with numerous plump fibroblasts and inflammatory cells were dominantly found in the midsubstance of the PL bundle, where collagen

507 fibers were loosely woven without the crimp pattern (Fig 3–C). (Original magnification
508 ×100)

Figure(s)

Figure(s)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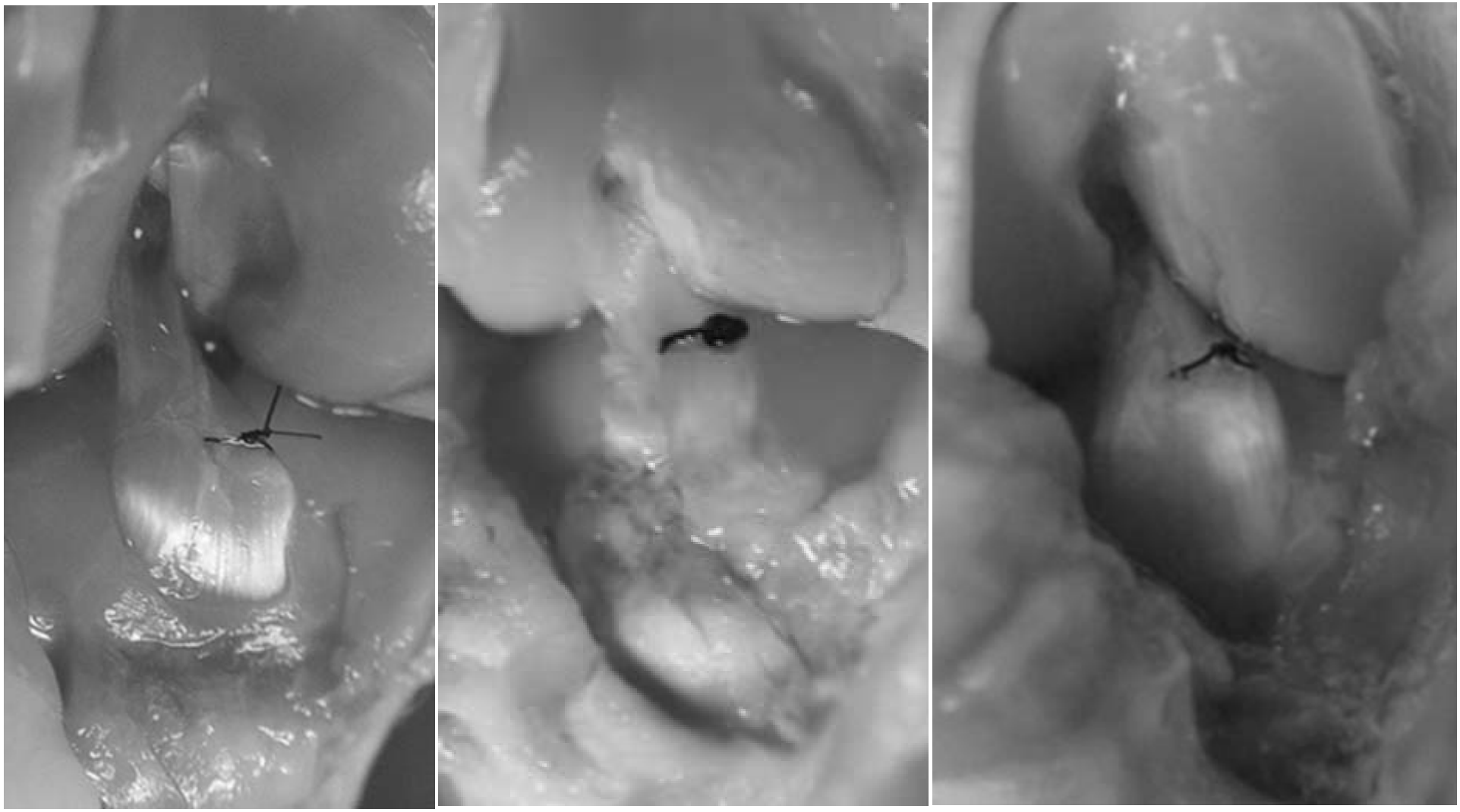
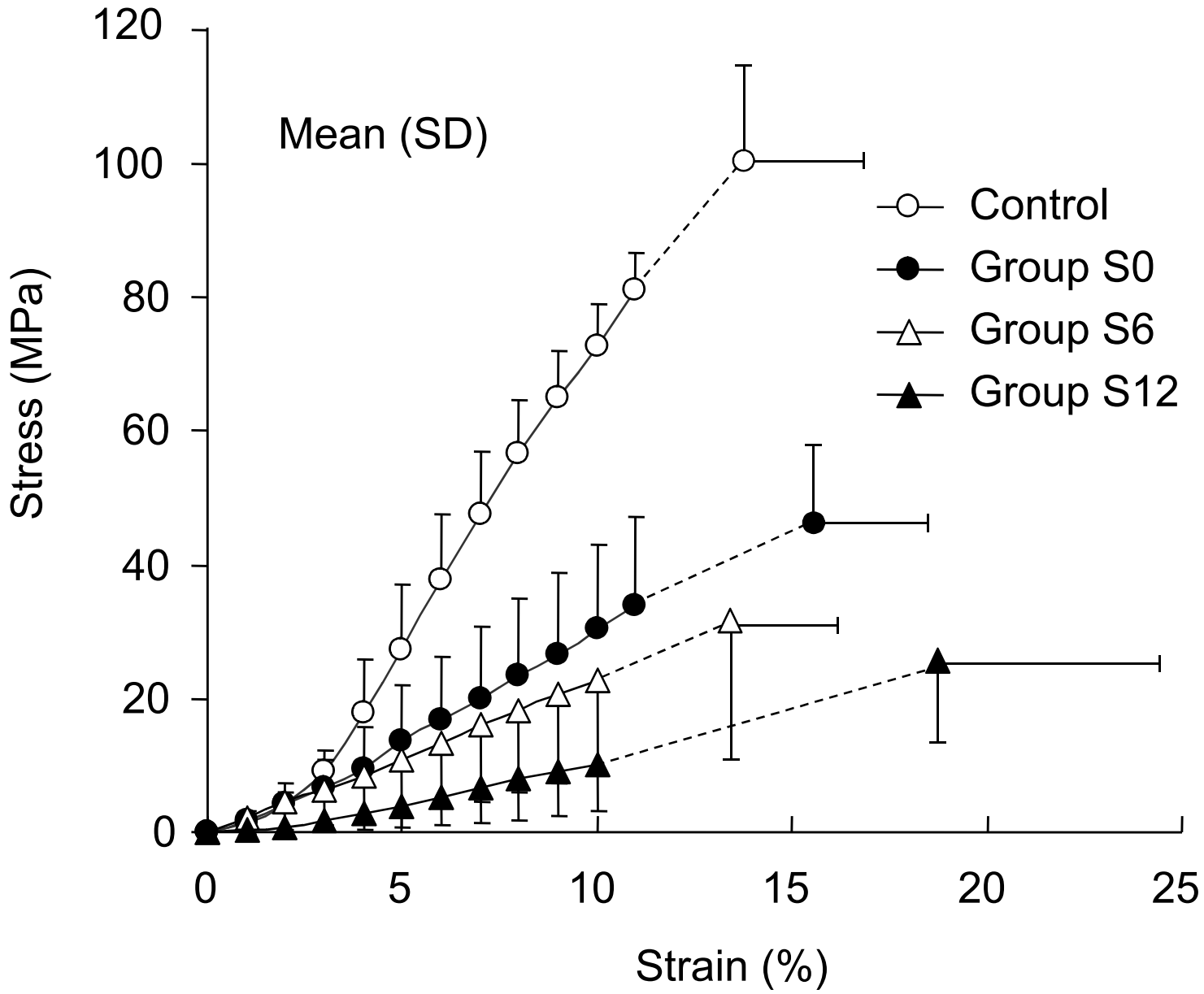


A B C

Figure(s)


Mean (SD)
Control
Group S0
Group S6
Group S12
Stress (MPa)
Strain (%)
0
20
40
60
80
100
120
0
5
10
15
20
25

Figure(s)

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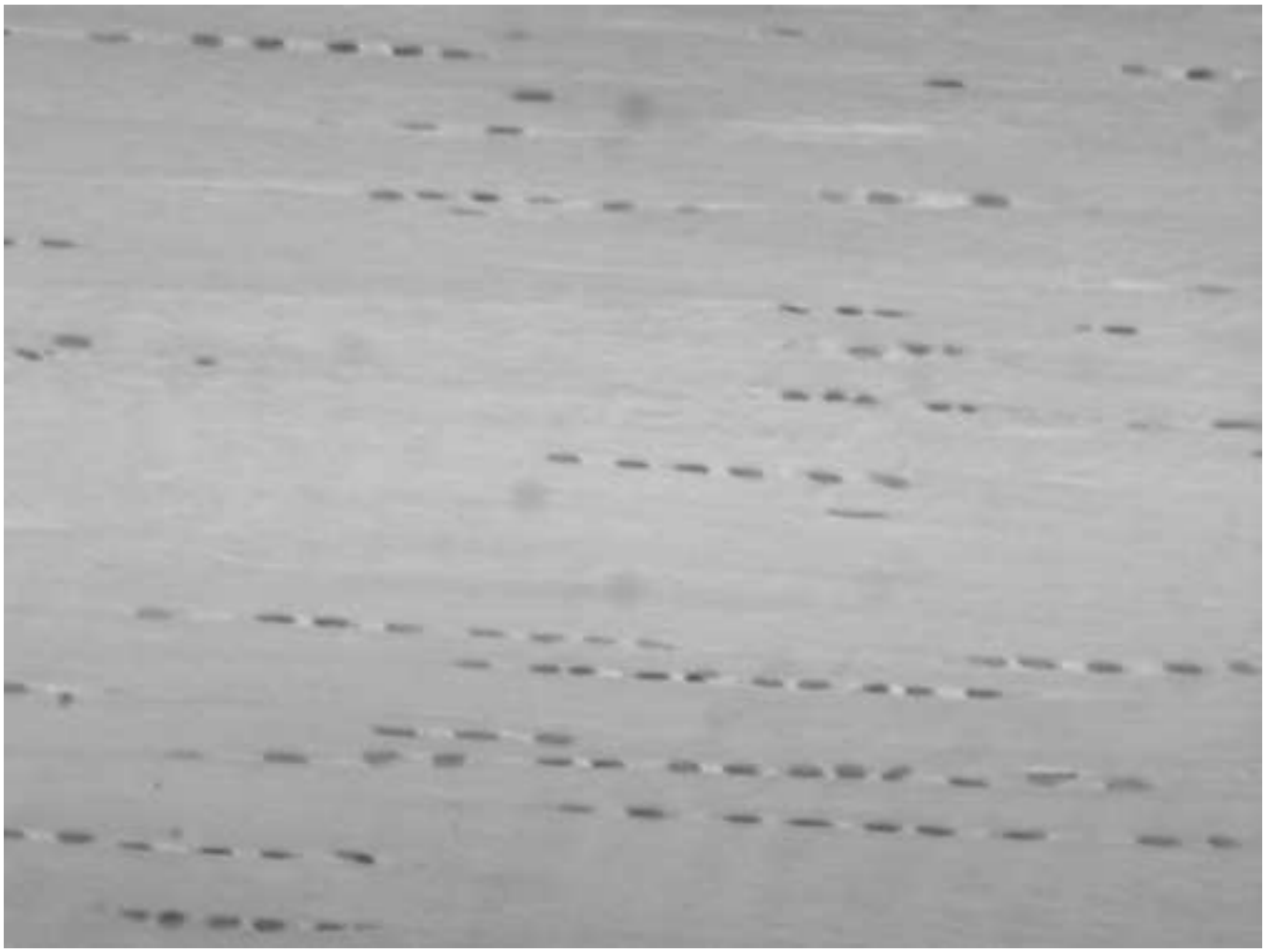

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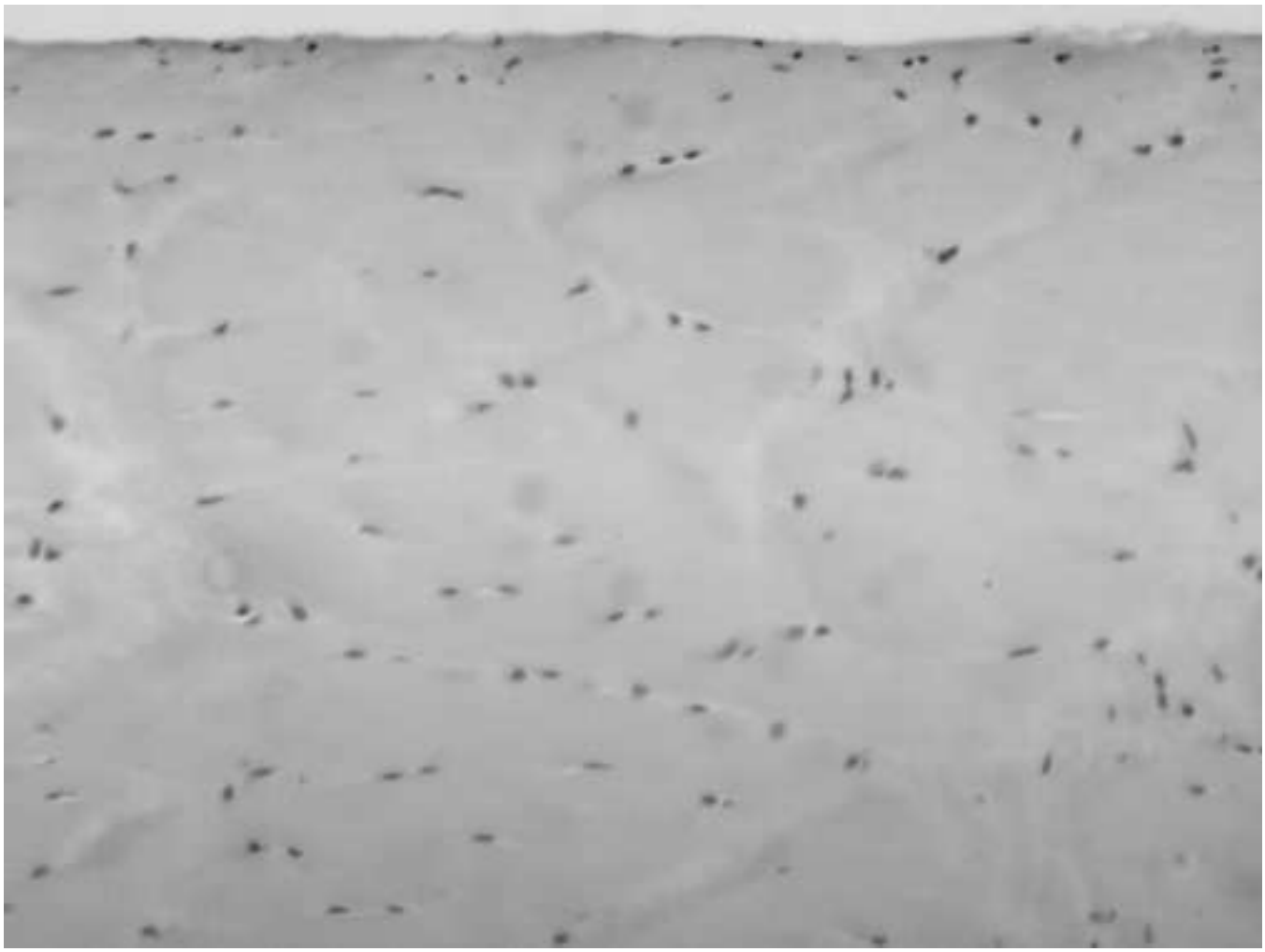

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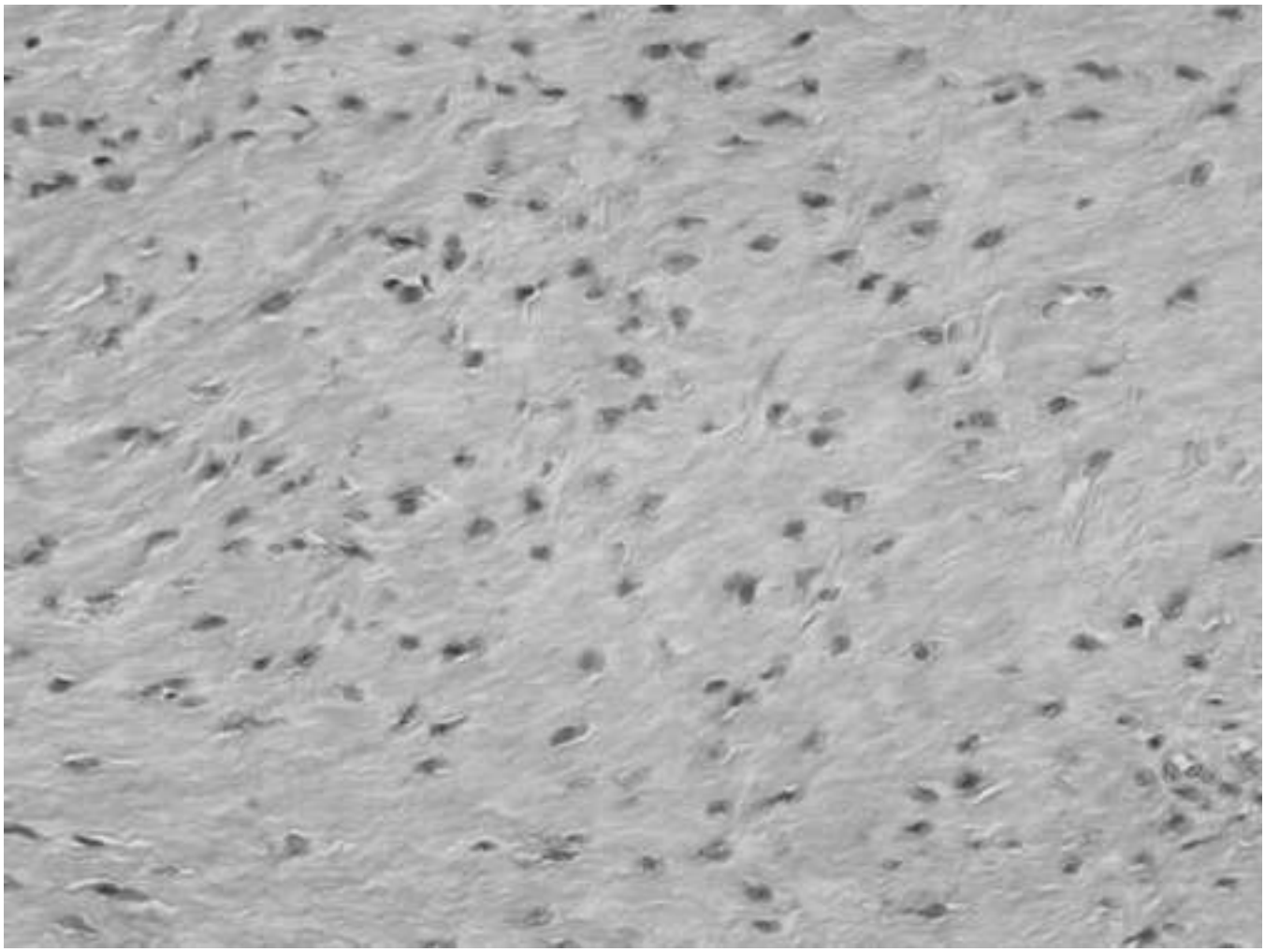

Table(s)

Table 1

The anterior-posterior translation of the knee and the tissue dimensions of the anterior cruciate ligament (Mean (SD))

Group	Anterior-posterior translation (mm)			ACL length	Cross-sectional area
	30º	60º	90º	(mm)	(mm^2)
Control	2.1 (0.5)	1.7 (0.3)	1.1 (0.3)	10.5 (0.6)	2.0 (0.2)
Group S0	1.4 (0.3) [a]	0.9 (0.2) [a]	0.5 (0.1) [a]	8.9 (0.3) [a]	2.2 (0.6)
Group S6	1.8 (0.1)	1.7 (0.2) [b]	1.0 (0.2) [b]	9.9 (1.7)	1.3 (0.6) [b]
GroupS12	2.0 (0.5) [b]	1.7 (0.3) [b]	1.1 (0.3) [b]	10.6 (0.7) [b]	1.7 (0.6) [b]

[a] Significantly different from the control group. ($p< 0.05$)

[b] Significantly different from the Group S0. ($p< 0.05$)

Table 2

Absolute values of the mechanical properties of the femur-PL bundle-tibia complex (Mean (SD)).

Group	Tensile strength (MPa)	Tangent modulus (MPa)	Strain at failure (%)
Control	101.6 (15.8)	886.3 (98.5)	13.8 (3.1)
Group S0	46.2 (11.3) [a]	373.2 (113.6) [a]	15.4 (2.8)
Group S6	30.6 (21.9) [a]	258.4 (168.7) [a]	13.4 (2.3)
Group S12	24.0 (12.4) [a, b]	158.2 (79.9) [a, b]	18.6 (6.3)

[a] Significantly different from the control group. ($p< 0.05$)

[b] Significantly different from Group S0. ($p< 0.05$)