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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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19 **Abstract**

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

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

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

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

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

45 **1. Introduction**

46 Radiofrequency (RF) energy has been clinically applied to shrinkage of
47 capsular tissues in the glenohumeral joint to restore joint stability (Abelow, 1997;
48 Fanton, 1998; Levy et al., 2001), based on *in vivo* and *in vitro* basic studies (Obrzut et
49 al., 1998; Hecht et al. 1998, 1999). Recently, several clinical reports have dealt with an
50 application of the RF energy to the anterior cruciate ligament (ACL) injuries (Carter et
51 al. 2002; Farnig et al., 2005; Halbrecht, 2005; Indelli et al., 2003; Perry and Higgins,
52 2000; Sekiya et al., 2000; Spahn and Schindler, 2002; Thabit, 1998). Some *in vitro*
53 studies, however, have been conducted to evaluate effects of RF energy on
54 biomechanical properties of the normal or injured ACL (Dodds et al., 2004; Kondo et al.
55 2005; Ma et al., 2005; Ozenci and Panjabi, 2003, 2005). Our previous *in vitro* study
56 (Kondo et al. 2005) demonstrated that the RF energy drastically reduces structural
57 properties of the ACL immediately after surgery, dependent of its magnitude, while the
58 RF energy significantly reduces the length of the ACL. Recently, a few *in vivo* studies
59 have shown that RF energy significantly deteriorates structural properties of the whole
60 ACL (Lopes and Markel, 2003; Scheffler et al., 2005). In the common shrinkage
61 treatment for the ACL, however, RF energy is applied to only the anterior aspect of the
62 ACL so that there are treated and intact portions in a cross-section of the ACL.
63 Therefore, the previous *in vivo* studies did not determine the mechanical properties of
64 the RF-treated lesion in the ACL, distinguishing from those of the untreated ligament
65 tissue.

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

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

76

77 **2. Methods**

78 ***2.1. Study Design***

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

93 device composed of a bipolar RF generator (Multi Electrode System 2000; Arthrocare,
94 Sunnyvale, CA, USA) and a probe (Arthrowand A1630-01; CAPSX, Arthrocare,
95 Sunnyvale, CA, USA) (Fig. 1). This probe contains 12 electrodes in an end having a
96 diameter of 3.0 mm. RF energy was set at a non-ablative level (Level 1; 28 Watts). This
97 level was chosen, because it is recommended for clinical usage. The RF treatment was
98 performed for 5 seconds, at each stroke, simulating clinical arthroscopic surgery using
99 the continuous small stroke technique (approximately 1 to 2 mm/seconds) (Hayashi et al.
100 1995). Briefly, a surgeon (E.K.) approached the midsubstance of the PL bundle with the
101 probe from only the anterior direction. Only the PL bundle was treated in physiological
102 saline solution at 90 degrees of knee flexion, because our pilot study showed that, when
103 the AM bundle was treated in the same manner, the AM bundle was completely torn at 6
104 weeks. The incised joint capsule and the skin wound were closed in layers with 3-0
105 nylon sutures, and an antiseptic spray dressing was applied. No immobilization was
106 applied after surgery. The animals were allowed unrestricted activities in their cages (52
107 cm in width, 35 cm in height, and 33 cm in depth). Then, all the animals were randomly
108 divided into 3 groups, Groups S0, S6, and S12, with 10 rabbits in each group. In these
109 groups, all animals were sacrificed at 0, 6, and 12 weeks after surgery, respectively. In
110 each group, 7 out of the 10 rabbits were used for biomechanical evaluation, and the
111 remaining 3 were used for histological observation with light microscopy. Nine knees
112 (Control group) randomly harvested from all left knees were used to obtain normal
113 control data.

114

115 ***2.2. Measurement of the anterior-posterior translation of the knee***

116 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 spray. 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

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

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

162

163 ***2.4. Tensile testing***

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

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

179

180 ***2.5. Histological observations***

181 In each limb intended for histological observation, the femur–PL bundle–tibia
182 complex was resected and fixed in a buffered 10 % formalin solution, decalcified, and
183 cast in paraffin blocks. For each block, we set a microtome so that the midsubstance
184 was sectioned parallel to the longitudinal axis. The central portion of the whole
185 specimen in the sagittal plane was marked on the block surface. The half of the
186 specimen was resected off until the central sagittal plane could be observed. Then, five
187 5- μ m continuous sections were obtained, and they were stained with hematoxylin and
188 eosin for histological observations. Two of the coauthors (KY, HT) observed the

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

192

193 **2.6. Statistical Analyses**

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

200

201 **3. Results**

202 **3.1. Anterior-posterior translation of the knee**

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

209

210 **3.2. Gross observation of inside the knee joint**

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

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

217

218 ***3.3. Tissue dimension of the posterolateral bundle of the ACL***

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

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

229

230 ***3.4. Mechanical properties of the posterolateral bundle of the ACL***

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

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

246

247 **3.5. Histology**

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

258

259 **4. Discussion**

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

274 The alternation that occurred in the mechanical properties of the RF treated
275 bundle tissue was well illustrated by the histological findings of this tissue. Immediately
276 after treatment, the tissue damage was characterized by the fused and homogenized
277 appearance of collagen tissues accompanied with the nuclear pyknosis of fibroblasts. At
278 6 and 12 weeks, granulation-like tissues with numerous plump fibroblasts were
279 predominantly found in the midsubstance of the ACL, where collagen fibers were
280 loosely woven without the crimp pattern. These histological changes observed in this
281 study are similar to those previously reported in the literature that dealt with the effect
282 of the thermal energy not only on the capsular tissues of the joint (Hecht et al. 1998,
283 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

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

311 Concerning the *in vivo* effects of RF treatment, Hecht et al. (1999) reported that
312 the stiffness of the joint capsular tissue that had been reduced by the RF treatment
313 returned to a normal value by 6 weeks. However, Lopez and Markel (2003)
314 demonstrated that the canine ACLs treated with monopolar RF energy were torn
315 approximately 55 days after treatment. In addition, Scheffler et al. (2005) reported the
316 effect of RF shrinkage on the structural properties of the elongated ACL in a sheep
317 model. They investigated that the initial reduction of knee laxity after RF treatment
318 could not be maintained at 24 weeks. A significant reduction in ultimate load was found
319 at 24 weeks in the RF-treated group compared with the untreated group. Schaefer et al.
320 (2005), who studied the *in vivo* response to laser thermal treatment in the rabbit patellar
321 tendon, reported that the treated tendons were stretched out within 4 weeks after
322 treatment. Wallace et al. (2002) stated that the thermal shrinkage to the relaxed medial
323 collateral ligament of the rabbit increased the creep deformation and the possibility of
324 failure by low physiologic stresses. In addition, it has been well known that healing
325 capacities of the ACL is lower than that of the extra-articular ligament and capsular
326 tissues (Amiel et al. 1990; Arnoczky et al. 1979) Therefore, we have to be pessimistic
327 concerning the early restoration of the mechanical properties of the ACL reduced by the
328 RF treatment.

329 As to clinical relevance of this study, recently, a few clinical studies dealing
330 with the electrothermal shrinkage for the elongated ACL have been reported. Thabit
331 (1996) treated 25 patients with the relaxed native ACL or the reconstructed ACL that

332 were lax but in continuity, using a RF device. They described that the KT-1000
333 arthrometer value was within 2 mm of that for the normal knee in 23 patients at the time
334 of 1.5 years or more after surgery. Spahn and Schindler (2002) also reported favorable 9
335 month follow-up results after RF treatment for 14 patients with secondary instability
336 after ACL reconstruction. Indelli et al. (2003) studied 28 patients with symptomatic
337 ACL laxity but with continuity of the ligament. After, monopolar RF treatment, 27 of
338 28 patients rated their knees as normal or nearly normal at minimum follow-up of
339 2-years and had KT-1000 side-to-side differences of less than or equal to 3 mm. On the
340 other hand, Carter et al. (2002) applied the electrothermal treatment for 18 patients who
341 had continuity of the ACL but had symptomatic laxity. However, a poor result was
342 obtained in 11 patients within several months after surgery. In addition, Perry and
343 Higgins (2000) reported a case of spontaneous, simultaneous rupture of both the ACL
344 and PCL 3 months after the electrothermal treatment under minimal physiologic load.
345 Sekiya et al. (2000) reported a case of autodigestion of the ACL after the electrothermal
346 treatment. Recently, Halbrecht (2005) reported the long-term failure of thermal
347 shrinkage for laxity of the ACL. 19 patients with partial tears of the ACL or stretched
348 ACL grafts underwent thermal shrinkage treatment using monopolar RF device. At
349 5-year follow-up, 11 of 13 patients had gone on to complete failure. They stated that
350 thermal shrinkage provides short-term benefit in the treatment of ACL laxity but leads
351 to catastrophic failure in the majority of patients at long term follow-up. Our study may
352 explain one of the causes of the poor results in these clinical reports. However, we have
353 to recognize that long-term follow-up studies have not been conducted with a sufficient
354 number of patients to clarify the clinical utility of the electrothermal treatment for the
355 ACL.

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

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

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

383

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483 **Figure legend**

484 Figure 1.

485 Operative photograph before the radiofrequency treatment.

486

487 Figure 2.

488 Apparatus for tensile testing

489

490 Figure 3–A, B, C.

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

494

495 Figure 4.

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

498

499 Figure 5-A,B,C.

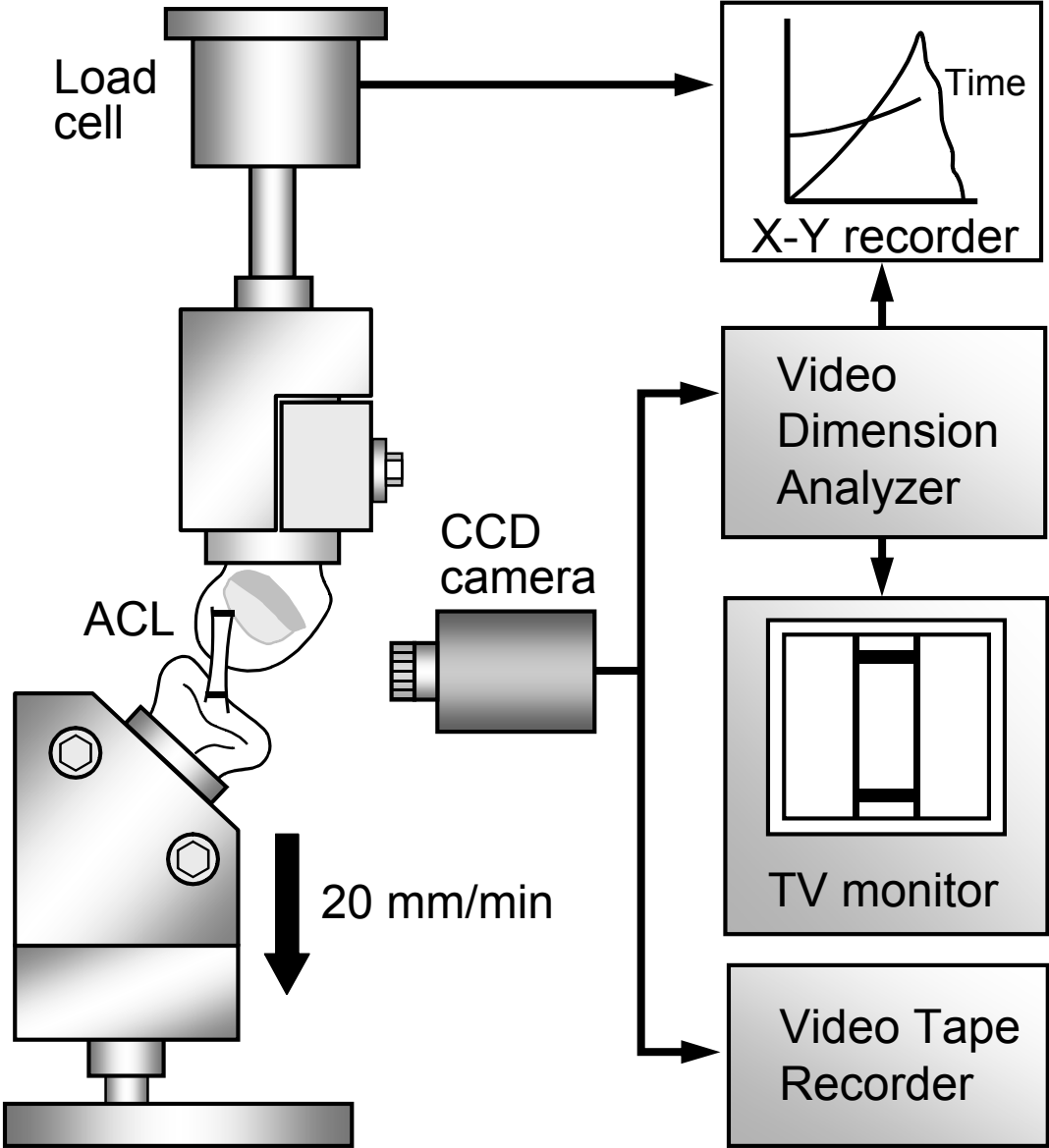
500 Histological findings in the midsubstance of the posterolateral bundle of the ACL with
501 light microscopy. **A:** Histology of the midsubstance of the normal ACL with light
502 microscopy. **B:** Histological examination performed immediately after the treatment
503 showed diffuse collagenous denaturation and pyknotic nuclear changes in fibroblasts in
504 the RF treated portion. The crimp patterns were not present in the treated area. **C:** In
505 Group S12, granulation–like tissues with numerous plump fibroblasts and inflammatory
506 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 $\times 100$)

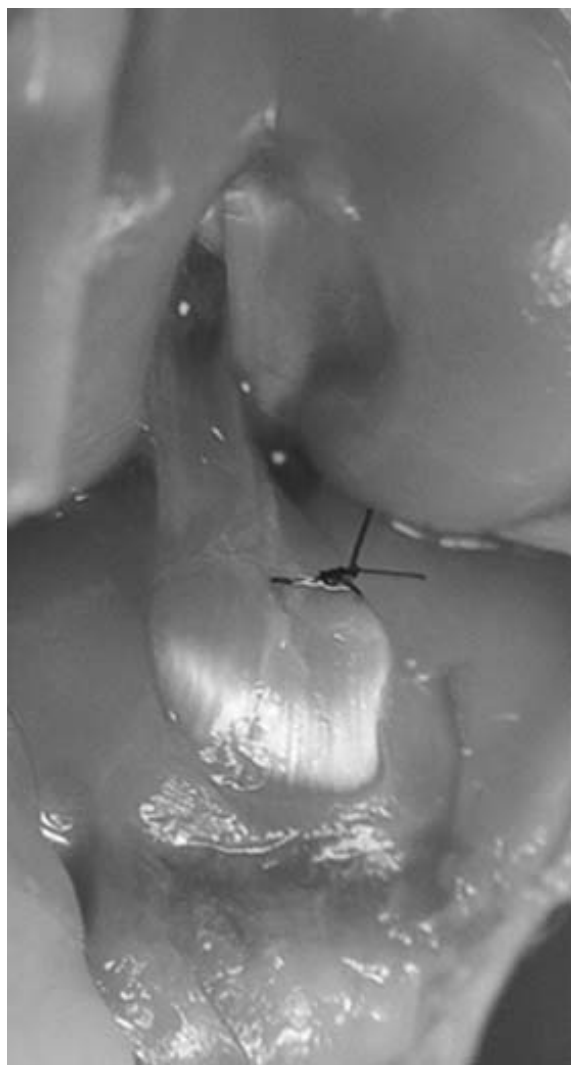
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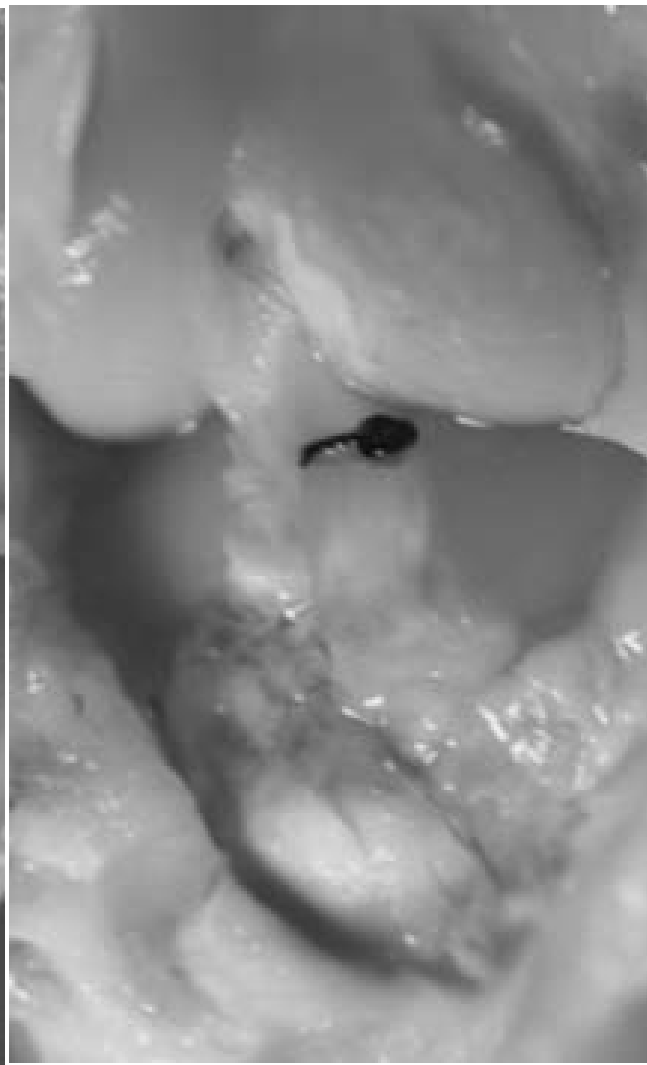
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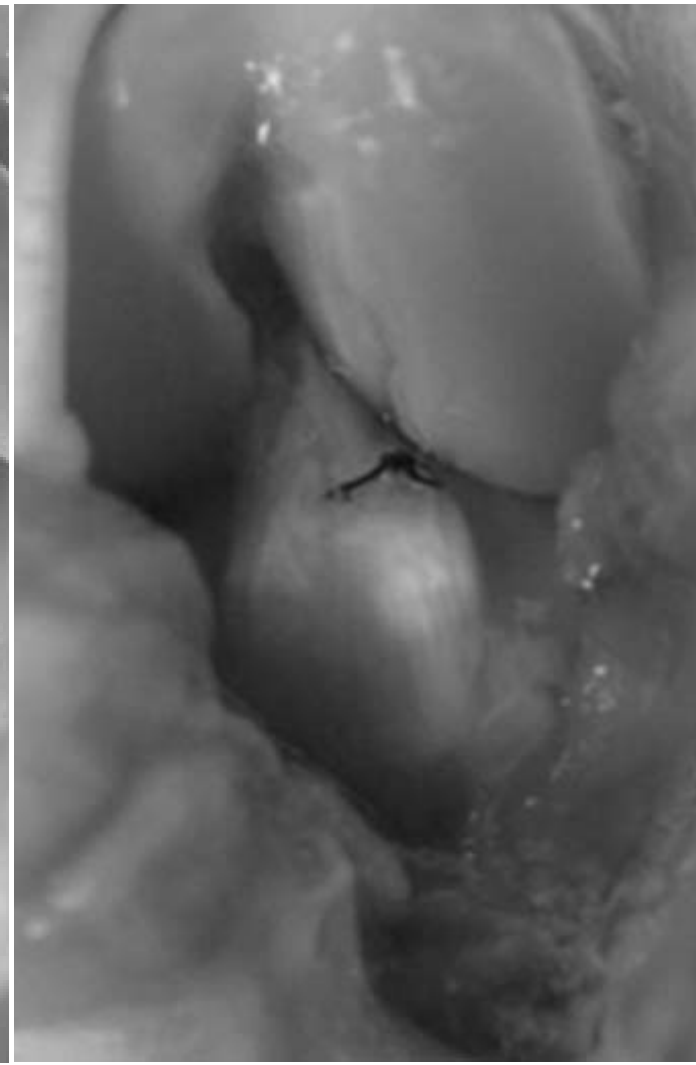
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A

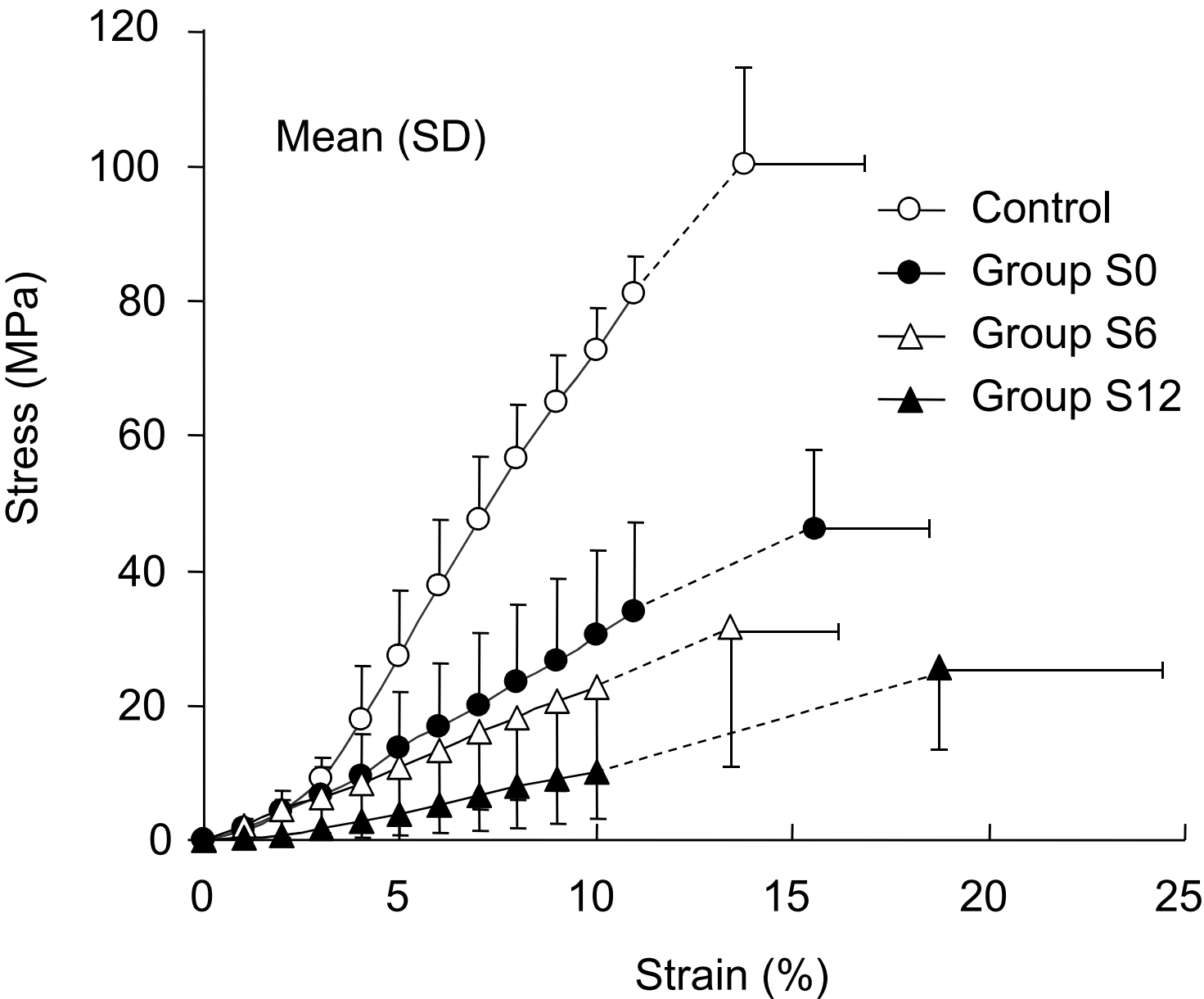


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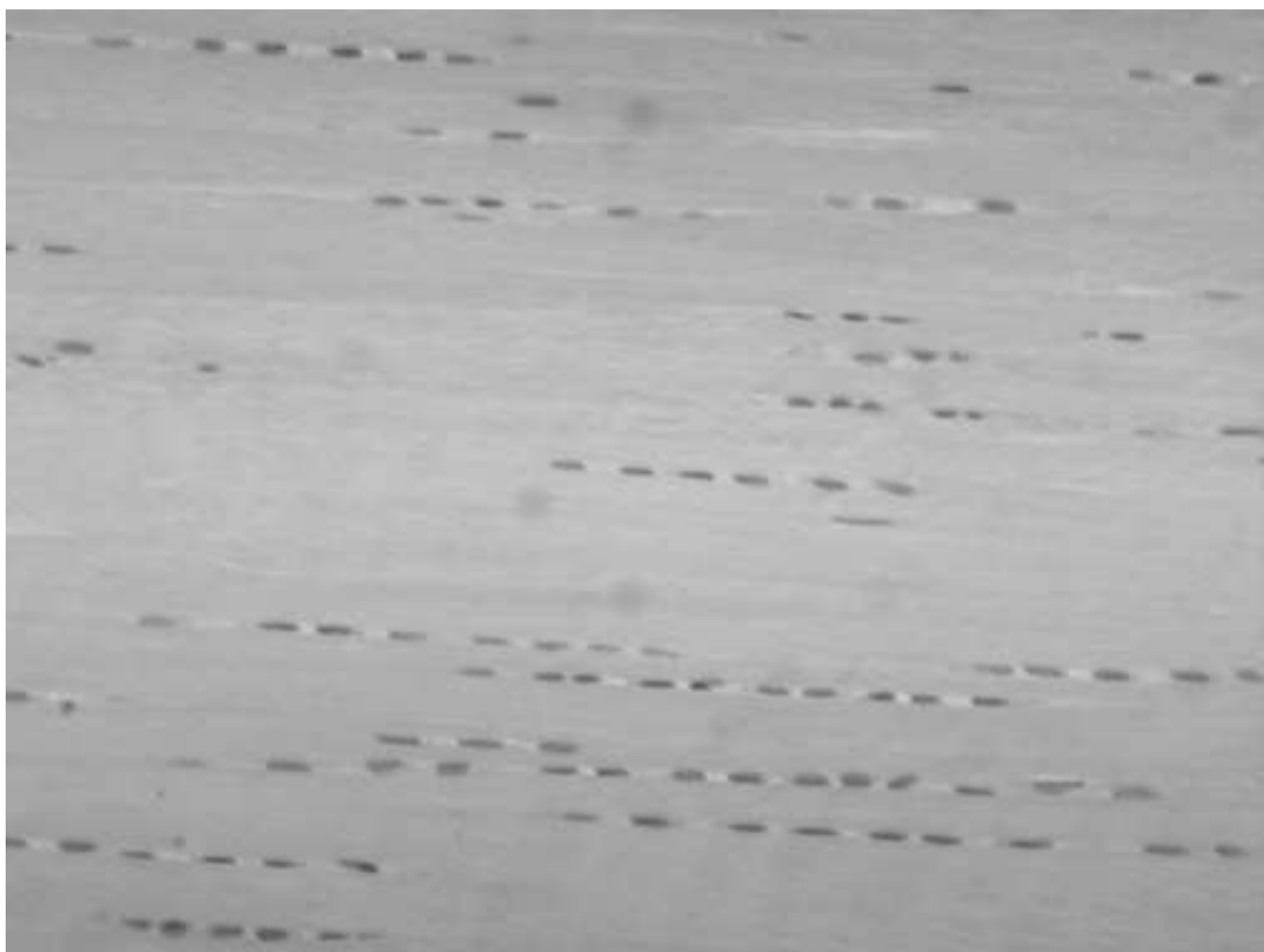


C

Figure(s)

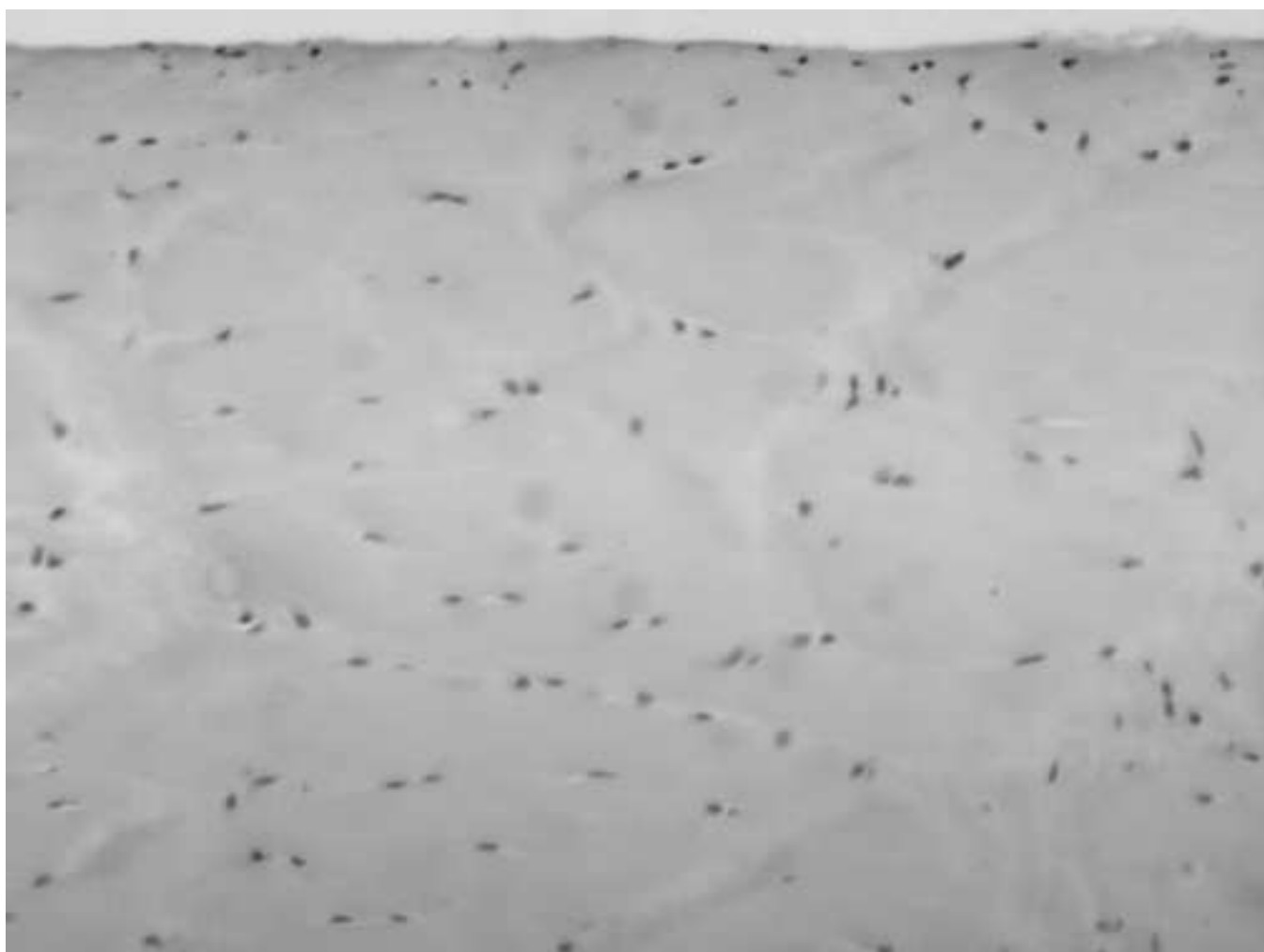


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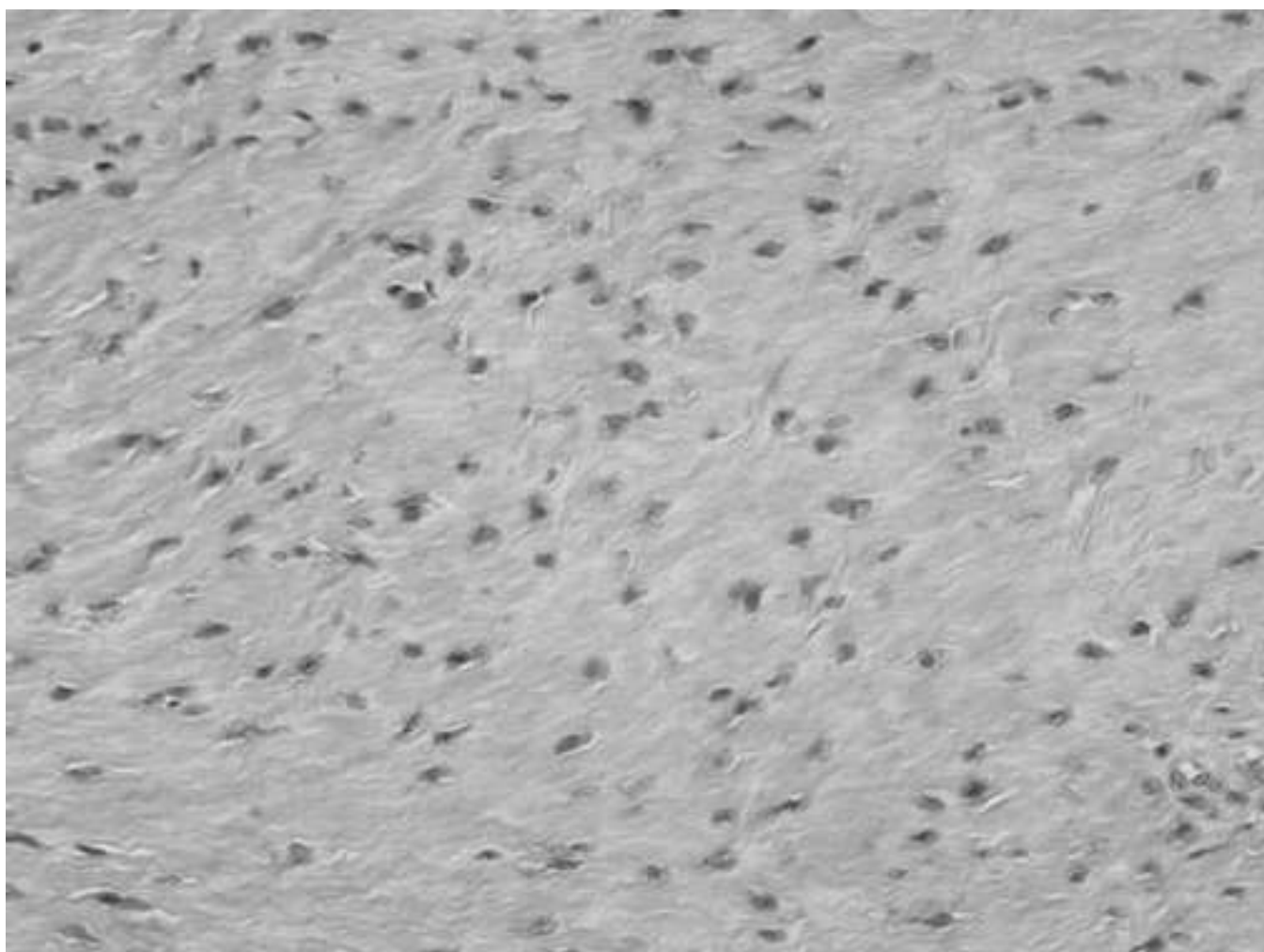


Table 1

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
	30°	60°	90°	(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. ($p < 0.05$)