Low-level laser therapy attenuates arthrogenic contracture induced by anterior cruciate ligament reconstruction surgery in rats

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Short title: LLLT attenuates ACLR-induced joint contracture

Summary

Therapeutic approaches to treat joint contracture after anterior cruciate ligament (ACL) reconstruction have not been established. Arthrofibrosis accompanied by joint inflammation following ACL reconstruction is a major cause of arthrogenic contracture. In this study, we examined whether anti-inflammatory treatment using low-level laser therapy (LLLT) can prevent ACL reconstruction-induced arthrogenic contracture. Rats underwent ACL transection and reconstruction surgery in their right knees. Unoperated left knees were used as controls. After surgery, rats were reared with or without daily LLLT (wavelength: 830 nm; power output: 150 mW; power density: 5 W/cm^2; for 120 s/day). We assessed the passive extension range of motion (ROM) after myotomy at one and two weeks post-surgery; the reduction in ROM represents the severity of arthrogenic contracture. ROM was markedly decreased by ACL reconstruction at both time points; however, LLLT partially attenuated the decrease in ROM. One week after ACL reconstruction, the gene expression of the proinflammatory cytokine *interleukin-1β* in the joint capsule was significantly upregulated, and this upregulation was significantly attenuated by LLLT. Fibrotic changes in the joint capsule, including upregulation of *collagen type I* and *III* genes, shortening of the synovium, and thickening were caused by ACL reconstruction and seen at both time points. LLLT

attenuated these fibrotic changes as well. Our results indicate that LLLT after ACL reconstruction could attenuate the formation of arthrogenic contracture through inhibition of inflammation and fibrosis in the joint capsule. Thus, LLLT may become a novel therapeutic approach for ACL reconstruction-induced joint contracture.

Keywords: anterior cruciate ligament reconstruction, low-level laser therapy, joint contracture, inflammation, arthrofibrosis

Introduction

Anterior cruciate ligament (ACL) injury, a major cause of sports-related incidents, induces joint instability [1,2]. The most common treatment for ACL injury is reconstruction surgery [1,2]. Although reconstruction surgery restores joint stability [2], one often-associated complication is joint contracture, which is characterized by reduction in range of motion (ROM) [3,4]. A systematic review by Wang et al showed that reported incidence rates of joint contractures after ACL reconstruction ranged from 0.1 to 71%, and the overall pooled incidence was 3% [5]. Joint contracture induced by ACL reconstruction causes knee pain and quadriceps muscle weakness [6,7], which disrupt the return to sports and daily activities [8,9]. Thus, prevention and/or improvement of joint contracture are critical issues in rehabilitation following ACL reconstruction.

In clinical practice, ROM exercises, continuous passive motions, surgical treatments, and manipulations under anesthesia are performed to treat ACL reconstruction-induced joint contractures. However, it has been reported that ROM exercises and continuous passive motions have limited or no effect on joint contracture [10-13]. Although surgical treatments and manipulations under anesthesia are effective in improving joint contracture, these treatments are linked to complications, such as

66 fractures, heterotrophic ossification, and cartilage damage [14]. Thus, the development
67 of alternative treatment strategies for ACL reconstruction-induced joint contracture is
68 necessary.

69 Both myogenic and arthrogenic factors contribute to the formation of joint
70 contracture after ACL reconstruction in both human patients [15-17] and rats [18,19]. In
71 70% of human patients who underwent surgical treatment for joint contracture after
72 ACL reconstruction, the formation was attributed to arthrofibrosis [20]. Therefore,
73 arthrofibrosis is an important target for joint contracture therapy. Peri-operative
74 inflammation is a major cause of arthrofibrosis [3,4,18-20]; thus, suppression of
75 inflammation may be an effective therapy against arthrofibrosis. Previous studies
76 reported that anti-inflammatory treatments using an interleukin-1 (IL-1) receptor
77 antagonist or corticosteroid improved ROM in patients with arthrofibrosis after ACL
78 reconstruction [21,22]. In clinical practice, however, these treatments are not widely
79 used due to the high cost and/or adverse effects.

80 To inhibit inflammation, we focused on low-level laser therapy (LLLT). LLLT
81 has anti-inflammatory and anti-fibrotic actions, and is associated with few adverse
82 effects [23-25]. Moreover, it is a low-cost therapy [24], and already widely used for
83 inflammatory and fibrotic diseases, such as arthritis and scarring [26,27]. In this study,

therefore, we aimed to examine whether LLLT can prevent ACL reconstruction-induced arthrogenic contracture via inhibition of inflammation. To achieve this, we examined the attenuative effects of LLLT on arthrogenic contracture, as well as inflammatory and fibrotic changes, using a rat model of ACL reconstruction.

Materials and Methods

Experimental animals

A schematic diagram of the experiment protocol is illustrated in Fig. 1. In this study, twenty-nine 8-week-old male Wistar rats (180–230 g; Japan SCL, Shizuoka, Japan) were used. Rats were randomly divided into ACL reconstruction (ACLR; n = 14) and ACL reconstruction plus LLLT (LLLT; n = 15) groups. Some data (i.e., ROM, synovial length, and joint capsule area) on the operated (right) side for all rats in the ACLR group were obtained from our previous study [19]. Experimental periods were set for one or two weeks (n = 7 or 8 rats/group/time point) post-operation, because inflammatory and fibrotic reactions after ACL reconstruction peak at one week and subside within two weeks [19]. Rats were housed in standard cages under controlled environment conditions (temperature of 20–25 °C, 12 h lighting cycle) with free access to standard rodent chow and water. Experimental procedures were approved by the animal

experimentation committee of Hiroshima International University (AE18-018).

ACL reconstruction surgery

We performed ACL reconstruction surgery on the right knees using previously described methods [18]. Rats were anesthetized with ketamine and xylazine (80 mg/kg and 10 mg/kg, respectively) by an intraperitoneal injection. The knee joint was opened via a medial parapatellar approach, and the ACL was transected. Using a 0.8 mm diameter Kirschner wire, bone tunnels were created from the antero-medial side of the proximal tibia to the lateral side of the distal femur. After passing the quadruple-bundle tail tendon autograft through the bone tunnels, both ends of the autograft were fixed to the bones using stainless steel interference screws (diameter of 0.8 mm and length of 2.0 mm, TE-00001; Matsumoto, Chiba, Japan). Finally, the joint capsule and skin were sutured. Unoperated left knees were used as controls. After surgery, the rats could move freely in the cage.

LLLT

After ACL reconstruction, rats in the LLLT group received daily LLLT using semiconductor laser systems (FINE LASER EL-800; Panasonic Healthcare, Tokyo,

Japan). Under ether anesthesia, LLLT was performed on the right knee under the conditions as follows: skin contact method, continuous irradiation mode, wavelength 830 nm, power output 150 mW, spot area 0.03 cm^2, power density 5 W/cm^2, attaching areas two points (medial and lateral sides of the knee), and irradiation time 60 s/point (Fig. 2). These irradiation conditions attenuate ACL reconstruction-induced joint swelling in the rat knee [28]. In addition, similar irradiation conditions (i.e., skin contact method, continuous irradiation mode, wavelength 830 nm, power output 100 mW, spot area 0.028 cm^2, power density 3.57 W/cm^2) could decrease inflammatory cytokines (IL-1β, IL-6, and tumor necrosis factor-α) in the articular cartilage in rat osteoarthritis model [29]. LLLT was started immediately after surgery and was performed every day until the day before sacrifice. Rats in the ACLR group did not receive any treatment after surgery.

Measurement of ROM

To assess the degree of arthrogenic contracture, we measured ROM after myotomy, which is determined by joint components, as previously described [30,31]. After each rat was sacrificed by exsanguination under ether anesthesia, the skin and knee flexor muscles were removed from the hindlimbs. Subsequently, the trunk and femur of the rat

placed in a spine position were fixed manually at a hip flexion of 90°. Then, the knee joint was extended by 14.6 N/mm extension moments, which stretch the rat knee joint close to its physiological limit but does not disrupt the joint components [32,33]. The angle between the femur and fibula was measured using a three-dimensional motion analysis system (Kinema Tracer; Kissei Comtec, Nagano, Japan) as ROM after myotomy. In a pilot study, we confirmed that ROM restriction is induced in the extension direction, but not in the flexion direction in our rat ACL reconstruction model at two weeks post-surgery (unpublished data). In this study, thus, ROM measurement was performed only in the extension direction.

Histological analysis

Tissue preparation

After ROM measurement, the knee joints were sampled and immersion-fixed in 0.1 M phosphate-buffered 4% paraformaldehyde (pH 7.4) at a flexion of 90° for two days at 4 °C. Next, samples were decalcified using 17.7% ethylenediaminetetraacetic acid (pH 7.2, Osteosoft; Merck Millipore, Darmstadt, Germany) for one month at room temperature and embedded in paraffin. Sagittal sections (thickness: 4 μm) were obtained from the medial midcondylar level.

156

157 ***Measurements of synovial length and joint capsule area***

158 The posterior region of the knee joint in the sections stained with aldehyde-fuchsin-
159 Masson-Goldner was photographed at 2× magnification. The superior and inferior
160 synovial lengths of the posterior joint capsule were measured according to previously
161 described methods [34] and summed as total synovial length. To assess joint capsule
162 thickening, the posterior joint capsule area was also measured according to previously
163 reported methods [34]. Measurements of synovial length and joint capsule area were
164 performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).
165 Posterior capsulotomy improves flexion contracture developed after ACL reconstruction
166 in human patients [35], implying that the posterior joint capsule is the structure
167 responsible for flexion contracture. In addition, fibrotic changes in the posterior joint
168 capsule were detected after ACL reconstruction in both human patients [17] and rats
169 [18,19,36]. In this study, thus, we focused on the posterior joint capsule.

170

171 ***Gene expression analysis***

172 Extraction of total RNA from the paraffin sections was performed as previously
173 described [37]. In brief, the posterior joint capsule was isolated from paraffin sections,

and total RNA was extracted using the RNeasy FFPE Kit (Qiagen, Hilden, Germany). Next, cDNA was synthesized using the total RNA and the SuperScript III First-strand synthesis system (Invitrogen, Grand Island, NY, USA).

Using the 7300 Real Time PCR System (Applied Biosystems, Foster City, CA, USA), real-time PCR was performed to quantify gene expression levels. TaqMan primer and probe sets for *IL-1β* (Rn00580432_m1), *type I collagen* (*COL1A1*; Rn01463848_m1), *type III collagen* (*COL3A1*; Rn01437681_m1), and *S18* (Rn01428913_gH) were obtained from Applied Biosystems. *S18* rRNA was used as the internal control. The calibration curve method was used to quantify gene expression levels.

Statistical analysis

The results were expressed as the mean ± standard deviation. Dr. SPSS II for Windows (SPSS Japan Inc., Tokyo, Japan) was used for statistical analyses. Two-way analysis of variance was used to examine the relationship between the intervention and time. If significant main or interaction effects were detected, post-hoc Bonferroni tests were used to localize the effects. If the interaction between time and intervention and the main effect of time were not significant, an unpaired t-test (if the normality assumption

had not been rejected by the Shapiro–Wilk test) or a Mann–Whitney test (if the normality assumption had been rejected by the Shapiro–Wilk test) with a Bonferroni adjustment was performed to compare differences between one and two weeks post-surgery. Differences were considered significant at P-values < 0.05.

Results

ROM

On the contralateral (left) side, ROM after myotomy was between 160° and 163° (Fig. 3). At one week post-surgery, ROM on the operated (right) side was 131 ± 11° and 150 ± 5° in the ACLR and LLLT groups, respectively. Two-way ANOVA revealed a significant main effect of intervention ($P < 0.001$). In both groups, ROM on the operated side was significantly smaller than that recorded on the contralateral side ($P < 0.001$). Between the operated sides, ROM was significantly larger in the LLLT group than in the ACLR group ($P < 0.001$). Similar results were obtained at two weeks post-surgery. ROM on the operated side was 137 ± 6° and 148 ± 6° in the ACLR and LLLT groups, respectively, and was significantly smaller than that observed on the contralateral side ($P \leq 0.003$). ROM on the operated side was significantly larger in the LLLT group than in the ACLR group ($P = 0.008$). The interaction between time and intervention (P =

0.156) and the main effect of time (P = 0.797) were not significant. There were no significant differences between one and two weeks post-surgery in all groups (P ≥ 0.508, unpaired t-test or Mann–Whitney test with a Bonferroni adjustment).

Synovial length

On the contralateral (left) side, the postero-superior joint space was blank, and the synovial membrane in the posterior joint capsule was deeply folded at both time points (Figs. 4a–d). At one week post-surgery, the postero-superior joint space was filled with fibrous tissue, and the synovial folds disappeared on the operated (right) side in the ACLR group (Fig. 4e). Two-way ANOVA revealed a significant main effect of intervention (P < 0.001). In the ACLR group, the total synovial length was significantly shorter on the operated side than on the contralateral side (P = 0.001) (Fig. 4i). On the operated side in the LLLT group (Fig. 4f), the postero-superior joint space and synovial folds remained largely unchanged, and the total synovial length was similar to that noted on the contralateral side (P = 0.634). At two weeks post-surgery, in both the ACLR (Fig. 4g) and LLLT (Fig. 4h) groups, the postero-superior joint space was filled with fibrous tissue, and the synovial folds were shallower on the operated side versus the unoperated left side. Consequently, the total synovial length was significantly

shorter on the operated side than on the contralateral side in both groups ($P \leq 0.007$). There were no significant differences in total synovial length between the operated sides in the ACLR and LLLT groups at either time point ($P \geq 0.307$). The interaction between time and intervention ($P = 0.592$) and the main effect of time ($P = 0.749$) were not significant. There were no significant differences between one and two weeks post-surgery in all groups ($P \geq 0.936$, unpaired t-test with a Bonferroni adjustment).

Joint capsule area

At one week post-surgery, in the ACLR group an apparent thickening of the posterior joint capsule was detected on the operated (right) side (Fig. 4e) compared with the contralateral (left) side (Fig. 4a). Two-way ANOVA revealed significant interaction between time and intervention ($P = 0.004$) and main effect of intervention ($P < 0.001$). The posterior joint capsule area was significantly enlarged compared with the area recorded for the contralateral side ($P < 0.001$) (Fig. 4j). Thickening of the posterior joint capsule was also observed on the operated side in the LLLT group (Fig. 4f). However, this thickening was milder than that noted on the operated side in the ACLR group. The posterior joint capsule area on the operated side in the LLLT group was also significantly larger than that on the contralateral side ($P < 0.001$), but significantly

246 smaller than the area determined for the ACLR group ($P < 0.001$). At two weeks post-
247 surgery, thickening of the posterior joint capsule on the operated side in the ACLR
248 group was partially attenuated (Fig. 4g). Consequently, the posterior joint capsule area
249 was significantly smaller than that measured at one week post-surgery ($P < 0.001$).
250 However, it remained significantly larger than that observed on the contralateral side (P
251 $= 0.001$). In the LLLT group (Fig. 4h), the posterior joint capsule area was significantly
252 larger on the operated side versus the contralateral side ($P < 0.001$), and comparable to
253 the area recorded for the ACLR group ($P = 1.000$). The main effect of time was not
254 significant ($P = 0.293$).
255
256 ***Gene expression***
257 In the expression of the inflammatory cytokine gene *IL-1β,* a significant main effect of
258 intervention was detected ($P = 0.045$), and significant simple main effects were detected
259 at only one week post-surgery. At one week post-surgery, the expression of the
260 inflammatory cytokine gene *IL-1β* in the ACLR group was significantly higher on the
261 operated side than on the contralateral side ($P = 0.013$) (Fig. 5a). The expression of *IL-*
262 *1β* on the operated side in the LLLT group was significantly lower than that measured in
263 the ACLR group ($P = 0.018$) and was similar to that recorded for the contralateral side

264 (P = 1.000). At two weeks post-surgery, the levels of *IL-1β* expression on the operated
265 side of the ACLR group returned to the levels observed for the contralateral side (P =
266 1.000). The interaction between time and intervention (P = 0.079) and the main effect of
267 time (P = 0.162) were not significant. Differences between one and two weeks post-
268 surgery were not significant in all groups (P ≥ 0.068, unpaired t-test or Mann–Whitney
269 test with a Bonferroni adjustment).

270 In the expression of *COL1A1,* a significant main effect of intervention was
271 detected (P < 0.001). At both time points, the expression of *COL1A1* in the ACLR group
272 was significantly upregulated on the operated side compared with the contralateral side
273 (P ≤ 0.003) (Fig. 5b). On the operated side in the LLLT group, *COL1A1* gene expression
274 was significantly lower than that measured in the ACLR group (P ≤ 0.042), and was not
275 significantly different from that determined for the contralateral side at both time points
276 (P ≥ 0.077). The interaction between time and intervention (P = 0.262) and the main
277 effect of time (P = 0.662) were not significant. Differences between one and two weeks
278 post-surgery were not significant in all groups (P ≥ 0.064, unpaired t-test or Mann–
279 Whitney test with a Bonferroni adjustment).

280 In the expression of *COL3A1,* a significant main effect of intervention was
281 detected (P < 0.001). At both time points, the expression of *COL3A1* on the operated

side was significantly higher than that recorded on the contralateral side in both ACLR and LLLT groups ($P \leq 0.011$) (Fig. 5c). Between the operated sides of the two groups, *COL3A1* gene expression was significantly lower in the LLLT group versus the ACLR group at one week post-surgery ($P < 0.001$); however, it was not significantly different at two weeks post-surgery ($P = 0.107$). The interaction between time and intervention ($P = 0.222$) and the main effect of time ($P = 0.231$) were not significant. There were no significant differences between one and two weeks post-surgery in all groups ($P \geq 0.984$, unpaired t-test or Mann–Whitney test with a Bonferroni adjustment).

Discussion

In this study, we examined whether LLLT can prevent ACL reconstruction-induced arthrogenic contracture. Our results indicate that LLLT can attenuate arthrogenic contracture via inhibition of inflammation and fibrosis in the joint capsule.

Inflammation stimulates the formation of arthrofibrosis, which is the most common cause of ACL reconstruction-induced joint contracture [3,4,18-20]. Thus, anti-inflammatory treatments may become a novel therapeutic strategy for the prevention of joint contracture after ACL reconstruction. In this study, we focused on LLLT as an anti-inflammatory therapy. The anti-inflammatory effects of LLLT have been reported in

300 both human and animal joints [23,25,27]. Our study corroborates these findings,
301 showing that LLLT downregulates the expression of the pro-inflammatory cytokine *IL-*
302 *1β* at one week post-surgery. IL-1 plays an important role in the formation of
303 arthrofibrosis. For example, intra-articular injection of the IL-1 antagonist anakinra
304 increases the ROM in patients with arthrofibrosis after ACL reconstruction [22].
305 LLLT might inhibit inflammation in the posterior knee joint capsule via direct
306 and indirect effects. A previous study reported that LLLT for cultured synoviocytes from
307 rheumatoid arthritis patients decreased expression of IL-1β at both the mRNA and
308 protein levels [38]. Thus, LLLT might inhibit inflammation by acting directly on the
309 cells in the posterior joint capsule. In addition, LLLT after injury has been shown to
310 inhibit inflammation and promote repair of the muscle [39] and bone [40], which are
311 damaged during ACL reconstruction surgery. It is considered that injured tissues can
312 lead to secondary damages in adjacent tissues through the release of inflammatory
313 cytokines. For example, exogenous inflammatory cytokines, such as IL-1β and tumor
314 necrosis factor-α, can induce inflammatory reactions in cultured human synoviocytes
315 [41,42]. Thus, the anti-inflammatory effects of LLLT on periarticular tissues other than
316 the posterior joint capsule might indirectly contribute to the inhibition of inflammation
317 in the posterior capsule.

Synovial shortening and joint capsule thickening are characteristic changes in arthrofibrosis, and are considered to be mechanisms of arthrogenic contracture after ACL reconstruction [17-19]. In this study, accordingly, synovial shortening and joint capsule thickening accompanied by upregulation of *COL1A1* and *COL3A1* expression levels were observed after ACL reconstruction in parallel with formation of arthrogenic contracture. LLLT after ACL reconstruction attenuated both synovial shortening and joint capsule thickening as well as the upregulation of the *COL1A1* and *COL3A1* genes at one week post-surgery. Therefore, the improvement in arthrogenic contracture as a result of LLLT can be explained, at least in part, by the inhibition of fibrosis in the joint capsule. However, at two weeks post-surgery, there were no differences in synovial length or joint capsule area on the operated side between the ACLR and LLLT groups. Arthrogenic contracture, represented by ROM restriction on the operated side, was significantly milder in the LLLT group versus the ACLR group. Thus, improvement in arthrogenic contracture by LLLT cannot be solely explained by the inhibition of fibrosis in the joint capsule. Apart from joint capsule fibrosis, osteoarthritis and cyclops syndrome may also contribute to ACL reconstruction-induced joint contracture [20]. Although we did not assess osteoarthritic changes, previous studies reported that LLLT could attenuate ACL transection-induced osteoarthritis [43-45].

The pathways leading joint contracture may be different between joint immobilization and our ACL reconstruction models. Our results suggest that inflammation and fibrosis pathways contributed to the formation of ACL reconstruction-induced arthrogenic contracture. Although inflammation and fibrosis in the joint capsule were also detected in the immobilized knee [46,47], anti-inflammatory treatments, including LLLT [48] and administration of non-steroidal anti-inflammatory drug celecoxib [49], could not attenuated immobilization-induced arthrogenic contracture. Thus, the contribution of inflammation and fibrosis pathways will be larger in joint contracture induced by ACL reconstruction than in that induced by joint immobilization.

The present study has some limitations. Firstly, most ACL reconstruction surgeries in patients are performed arthroscopically [50], but we selected open surgery. Nevertheless, the effect of open surgery on increasing the risk of joint contracture remains controversial [51,52]. Thus, contractures observed in this study may have been overestimated compared with those observed following arthroscopic surgery. However, we previously revealed that arthrotomy (i.e., opening of the joint capsule) alone did not reduce ROM after myotomy under our experimental conditions [36]. Secondly, the follow-up periods were relatively short (up to two weeks). Additional long-term studies are warranted to confirm the favorable effects of LLLT on joint contracture. Thirdly, rats

in ACLR group did not undergo daily anesthesia. Between the ACLR and LLLT groups, however, there were no differences in all parameters on the contralateral side. Thus, we consider that effects of anesthesia on contracture formation were negligible. Fourthly, we used young rats (eight-week-old) for the experiment, because ACL reconstruction surgery in pediatric and the adolescent patients has steadily increased [53]. The effect of age on the ACL reconstruction-induced joint contracture remains controversial [54-56], and we cannot exclude the possibility that different results are obtained from older rats.

In conclusion, LLLT after ACL reconstruction could attenuate the formation of arthrogenic contracture through inhibition of inflammation and fibrosis in the joint capsule. Thus, LLLT may be a novel, safe, and effective therapeutic approach for treating ACL reconstruction-induced joint contracture.

Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

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583 **Figures**

Fig 1

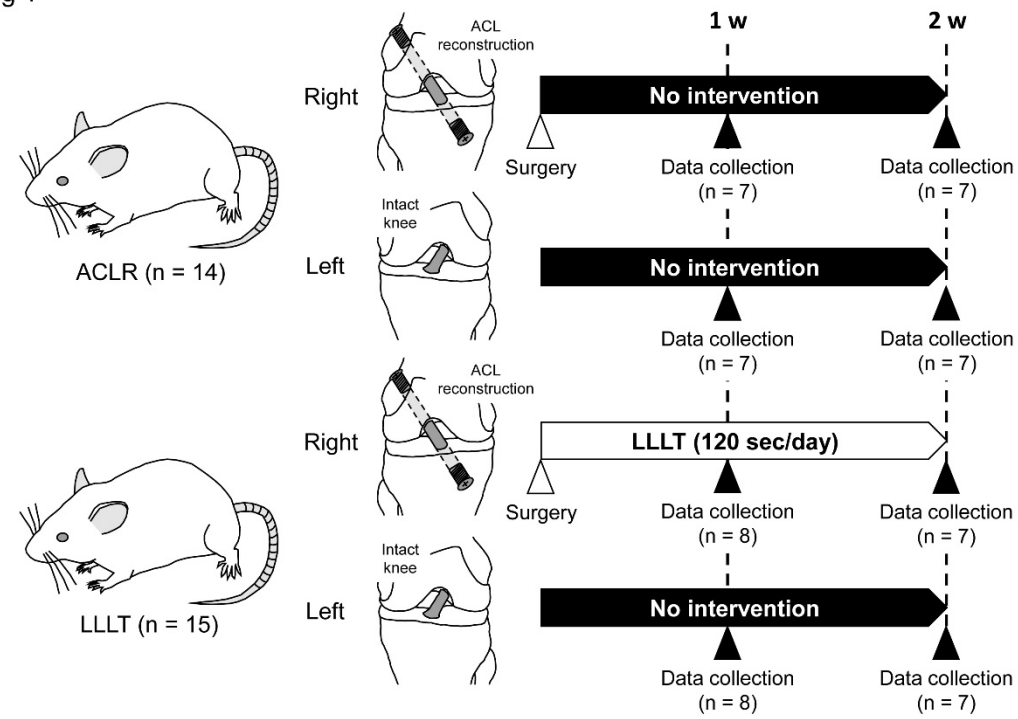

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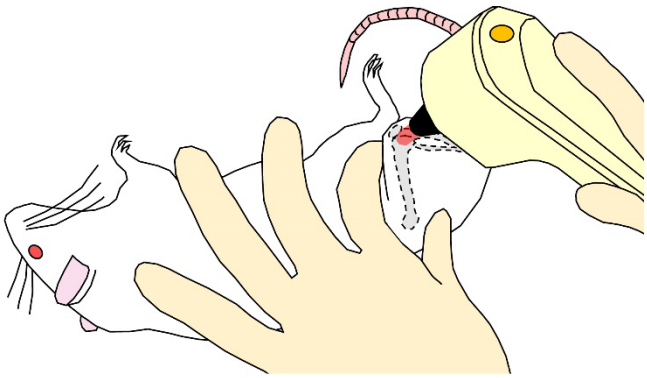
Fig 2



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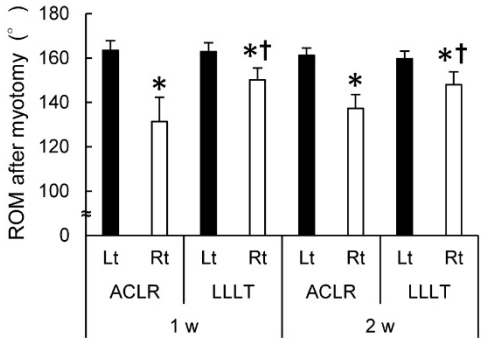

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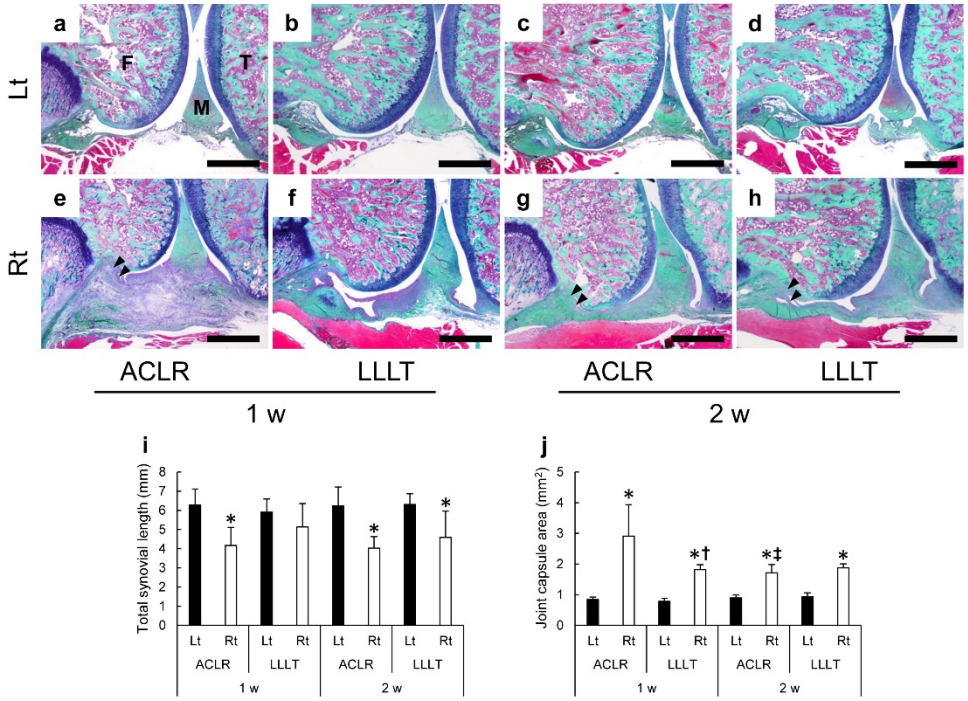

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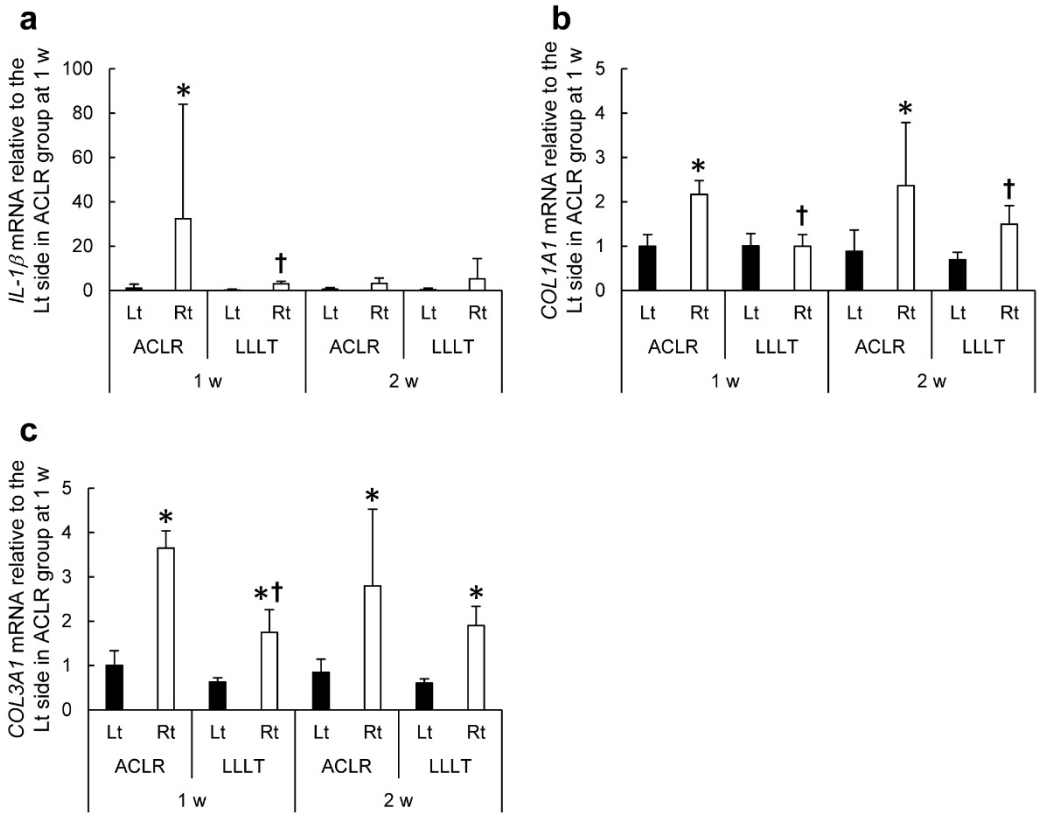
Fig 5



588

589 **Figure legends**

590 Figure 1. Experimental protocol. ACLR, anterior cruciate ligament reconstruction;

591 ACL, anterior cruciate ligament; LLLT, low-level laser therapy.

592

593 Figure 2. Image of LLLT. LLLT was applied to the medial and lateral sides of the knee

594 joint. LLLT, low-level laser therapy.

595

596 Figure 3. ROM after myotomy. Values are shown as the mean and standard deviation. *,

597 significant difference compared with the contralateral side ($P < 0.05$). †, significant
598 difference compared with the same side in the ACLR group at the same time point ($P <$
599 0.05). ACLR, anterior cruciate ligament reconstruction; LLLT, low-level laser therapy;
600 Lt, left; Rt, right; ROM, range of motion.
601
602 Figure 4. Histomorphometric changes in the posterior knee joint capsule. Representative
603 images of the aldehyde-fuchsin-Masson-Goldner-stained posterior knee joint in the
604 ACLR group at one week (a and e), LLLT group at one week (b and f), ACLR group at
605 two weeks (c and g), and LLLT group at two weeks (d and h). (a–d) and (e–h) show the
606 contralateral (Lt) and operated (Rt) sides, respectively. Arrowheads indicate the postero-
607 superior joint space filled with fibrous tissue. Scale bars = 1 mm. (i) Total synovial
608 length. (j) Joint capsule area. Values are shown as the mean and standard deviation. *,
609 significant difference compared with the contralateral side ($P < 0.05$). †, significant
610 difference compared with the same side in the ACLR group at the same time point ($P <$
611 0.05). ‡, significant difference compared with the same group at one week ($P < 0.05$).
612 ACLR, anterior cruciate ligament reconstruction; LLLT, low-level laser therapy; Lt, left;
613 Rt, right; F: femur, T: tibia; M: meniscus.
614

615 Figure 5. Gene expression levels in the posterior joint capsule. (a) *IL-1β*, (b) *COL1A1*,

616 and (c) *COL3A1*. Values are shown as the mean and standard deviation. *, significant

617 difference compared with the contralateral side ($P < 0.05$). †, significant difference

618 compared with the same side in the ACLR group at the same time point ($P < 0.05$).

619 ACLR, anterior cruciate ligament reconstruction; LLLT, low-level laser therapy; Lt, left;

620 Rt, right; *IL-1β, interleukin-1β*; *COL1A1*, *type I collagen*; *COL3A1*, *type III collagen.*