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Effects of a Therapeutic Laser on the Ultrastructural Morphology of Repairing Medial Collateral Ligament in a Rat Model

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Background and Objectives: Low energy laser therapy has been shown to enhance mechanical strength of healing medial collateral ligament (MCL) in rats. The present study investigated its effects on the ultrastructural morphology and collagen fibril profile of healing MCL in rats.

Study Design/Materials and Methods: Thirty-two mature male Sprague–Dawley (SD) rats were used. Twenty-four underwent surgical transection to their right MCLs and eight received only skin wound. Immediately after surgery, eight of the MCL transected rats were treated with a single dose of laser therapy at 63.2 J cm^{-2}, eight were treated with a single dose of laser therapy at 31.6 J cm^{-2}, the rest had no treatment and served as control. At 3 and 6 weeks after surgery, the MCLs were harvested and examined with electron microscopy for collagen fibril size, distribution, and alignment.

Results: Significant differences ($P < 0.001$) were found in fibril diameters from the same anatomical site and time period among different groups. The mass-averaged diameters of the laser-treated (64.99–186.29 nm) and sham (64.74–204.34 nm) groups were larger than the control group (58.66–85.89 nm). The collagen fibrils occupied 42.55–59.78, 42.63–53.94, and 36.92–71.64% of the total cross-sectional areas in the laser-treated, control and sham groups, respectively. Mode obliquity was 0.53–0.84 among the three groups.

Conclusions: Single application of low energy laser therapy increases the collagen fibril size of healing MCLs in rats. Lasers Surg. Med. 32:286–293, 2003.
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Key words: healing; injury; collagen fibril; laser therapy; rat

INTRODUCTION

Low level laser therapy (LLLT) is a popular choice of treatment for musculoskeletal conditions [1–7]. In our recent study [8], we reported that LLLT improved the biomechanical strength of healing ligaments in vivo at both 3 and 6 weeks post-injury.

In order to understand the mechanism underlying the therapeutic effects of LLLT, it is necessary to study the change in tissue histology with this treatment. Although studies have been done to examine the effects of LLLT on fibroblastic activity and collagen content [9–11], very few have thoroughly examined its effects on the collagen fibril architecture in different anatomical sites of a ligament [12]. Besides collagen fibril size, the fibril density and alignment, which contribute to the biomechanical functions of ligaments and tendons [13], have not been reported.

Since the late 1970s, studies have been done to investigate the ultrastructural morphology of collagen fibrils with electron microscopy and image analysis techniques [14–23]. Reports on the correlation between biomechanical strength and fibril size of connective tissues are equivocal [14,16,23]. In the study by Binkley and Peat [14], alteration of collagen fibril size was found with deterioration of the structural stiffness and ultimate tensile stress of the tissue. Frank et al. [16] did not find any correlation between the mechanical properties and collagen fibril diameters of healing medial collateral ligaments (MCLs) in rabbits. In a study by Reddy et al. [23], evidence of increase in collagen synthesis and remodeling of mature collagen was found in healing tendons, although their biomechanical performances were still inferior to the normal tendons. In our recent study [8], we found that transected MCLs treated with a laser therapy had better biomechanical strength than those without treatment. The ultimate tensile strength (UTS) of LLLT treated MCLs were comparable to those of uninjured MCLs as early as 3 weeks after injury, which might be partly due to an increased collagen synthesis of the repairing ligament [11]. However, we believe that collagen synthesis may only be one of the changes induced by LLLT, and there could be other changes such as fibril diameters, distributions, and orientations with laser treatment. Therefore, we tested the effect of LLLT on the collagen ultrastructural morphology of healing MCLs of rats at both 3 and 6 weeks post-injury.

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MATERIALS AND METHODS

Thirty-two mature male Sprague–Dawley (SD) rats with mean weight of 310 g (range: 285–352 g) aged 12 weeks at the time of surgery were used for the study. The Animal Subjects Ethics Subcommittee of our university approved this study. The animals were randomly allocated into eight groups as shown in Table 1.

Surgery and Treatment

Both the surgical and treatment procedures were similar to our previous study [8]. In brief, surgeries were carried out under general anesthesia with intra-peritoneal injection of a mixture of 100 mg/ml ketamine (Alfasan International, Woerden, the Netherlands) and 20 mg/ml xylazine (Alfasan International). The dosages of the drugs applied were calculated with a ratio of 8 mg ketamine/0.8 mg xylazine per 100 g of the animal's weight. For the experimental groups, MCL of the right knee was exposed and completely transected with a scalpel at the middle portion. A probe with a divergence adapter that produces gallium–aluminum–arsenide (GaAlAs) laser beam of wavelength 660 nm and average power output 8.8 mW was applied directly on the transected MCL immediately after injury. This laser beam was a pulsed square wave of 10 kHz with a circular irradiation area of 0.125 cm^2. The average power output of this pulsed laser unit was calibrated with a power meter prior to the study to ensure consistency of output. The duration of laser treatment was 15 minutes for group Laser15 and 7.5 minutes for group Laser7.5 (Table 1). Knowing the power output and the laser irradiation area, the energy density for the groups Laser15 and Laser7.5 could be calculated based on the formula (Energy density = average power × time/area) [9,12] and the results were 63.2 and 31.6 J cm^{-2}, respectively. Since these testing energy densities (63.2 and 31.6 J cm^{-2}) were calculated according to the power output (8.8 mW) recorded at the 10 KHz pulsed mode, the energy densities were therefore the resultant of this pulsed laser.

For the control animals, the surgical procedure was the same as the experimental groups, but only placebo laser treatment was applied to the MCL for 7.5 minutes without turning on the power. For the sham group, only skin wound was induced but the MCL was not transected and no laser therapy was applied. All the skin wounds were sutured immediately after treatment. The rats were kept in cages with unlimited activities inside a room with a 12-hour day light cycle. The light source was from a 100 W full spectrum light bulb of 158 lux. In order to standardize the exposure to the light source, all the rats were kept about 2.4 m away from it. Food and water was given ad libitum.

Tissue Processing

The rats were euthanized either at 3 or 6 weeks post-surgery by double dose injection of anesthetics. The right MCLs were excised with a small bone chip attached at the femoral insertion for recognition of the MCL orientation, immediately fixed in a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde, and postfixed in osmium tetroxide for 2 hours. The specimens were then processed in graded alcohol for dehydration and embedded in epoxy resin. Ultra-thin sections of about 90 nm were cut perpendicularly to the long axis of the ligament with an Ultracut E ultramicrotome (Reichert-Jung, Vienna, Austria) at the femoral end, middle, and tibial end of the ligament. The ultra-thin sections were mounted on 200-mesh copper grids, stained with uranyl acetate and lead citrate, and then examined in a Philips 208s transmission electron microscope (TEM) (Philips Electron Optics, Eindhoven, the Netherlands), operated at 80 kV.

TEM Sampling and Image Analysis

Assuming the cross section of MCL is regular in shape, the portions immediately adjacent to the paraligament would be considered as peripheral, and those fell within half of the distance from the center point to the paraligament were considered as the core. Four images of magnification of 32,000 times were taken from the core and peripheral regions of each section using a selective random sampling method similarly to Frank et al. [15]. Areas containing cells or fracture artefacts were avoided during photography. Due care was taken to maintain the conditions and magnification factors throughout the entire sampling process in order to avoid any variations in the measurements for tissues from different sites and groups. Variations in the magnification inherent to the electron microscope were minimized by normalizing the electron beam before each TEM examination. Furthermore, before and after each TEM filming session, the magnification was

TABLE 1. Experimental, Control, and Sham Groups of the Study

Group (n = 4)	Surgery (right MCL)	Laser treatment	Time of sacrifice (weeks after surgery)
Laser15	Transected	15 minutes	3
Laser7.5	Transected	7.5 minutes	3
Control	Transected	Placebo	3
Sham	Exposed only	No	3
Laser15	Transected	15 minutes	6
Laser7.5	Transected	7.5 minutes	6
Control	Transected	Placecebo	6
Sham	Exposed only	No	6

calibrated with a carbon cross-grafting replica grid of size 2,160 line/mm (Polysciences Incorporation, Warrington). The calibration procedure provided an estimate of the total sample area to an accuracy of within 2.0%.

The TEM images were later analyzed by an image analysis system (analySIS, Soft Imaging System, GmbH, Leinfelden-Echterdingen, Germany) following a standard procedure [24]. This process involved an interactive program for identification of individual collagen fibrils and discrimination of these fibrils from the background. An automated sequencer incorporated in the program measured the minimum fibril diameter, fibril cross-sectional area, and obliquity.

Having collected the data of each measured collagen fibril, descriptive statistics including the fibril density and mean minimum fibril diameter were calculated. Due to heterogeneity in fibril size, fibrils with extreme diameters do not reflect their actual contribution to the cross-sectional area of the ligament [25], therefore percentage area covered by fibrils was calculated. This was done by calculating the sum of the cross-sectional areas of all the measured fibrils and expressed it as a percentage of the total measured area of the MCL.

Another parameter known as mass-averaged diameter was calculated. Mathematically, it is expressed as $\Sigma nd^3/\Sigma nd^2$, where n is the number of measurements made for fibrils of diameter d [26]. The mass-averaged diameter is an index that reflects the contribution of some large diameter fibrils to the mass of the ligament. It is revealed in the formula that the size factor has a cubic weighting over its number factor in determining the mass-averaged diameter.

In order to further investigate the size distribution of the fibril on the same MCL site, the fibrils were categorized into ten classes according to their minimum diameters that ranged from 0 nm to above 500 nm (50 nm/class) to represent the fibril size distribution pattern.

Besides fibril size, the obliquity factor was also measured. Obliquity was calculated by comparing the minimum diameter of each fibril with its maximum diameter. Assuming that each collagen fibril is a cylindrical structure, the more circular the cross-sectional shape suggests that the fibril is more perpendicular to the direction of cutting. The obliquity factor has a range of 0–1 and the values are indicative of the circularity of the cross-sectional shape of the fibrils. The mode obliquity values were calculated to represent the level of orientation of the fibrils.

The above measurements were performed for all the six anatomical sites. The collagen fibril population profiles of the different sites were compared using a non-parametric Kruskal–Wallis test [27] with α set at 0.05. Further pairwise comparison was done using Mann–Whitney U-test to identify the data pairs that were significantly different [27].

RESULTS

On visual examination, all the repairing MCLs showed signs of repair with thick fibrous scar formation. The MCLs in the laser-treated and control groups appeared to be larger than that of the sham group. The anatomical orientations of all the MCLs were comparable at the macroscopic level.

All specimens had similar appearance on the TEM micrographs with large clusters of collagen fibrils surrounding the cells. Each micrograph contained about 200–1,200 fibrils (Figs. 1 and 2a). Generally, micrographs taken from the peripheral regions of all groups contained predominantly small fibrils (Fig. 1), whereas for the core regions, both laser-treated groups had slightly more large fibrils than the control group (Fig. 2a–c). The pattern between the two laser-treated groups were quite similar (Fig. 2a,b). A very distinct pattern of large diameter fibrils interlaced with small fibrils was found in the core regions of the sham group (Fig. 2d). The fibril distributions appeared to be similar between the 3- and 6-week groups.

A total of 399,817 fibrils were analyzed in this study. Both the laser-treated and control groups had more number of fibrils per cross-sectional area than the sham group (Table 2). However, the actual percentage of area covered by collagen fibrils were quite similar among groups (Table 3). There is not much difference in fibril density or fibril coverage of the same site between the 3- and 6-week time frames (Tables 2 and 3).

The mass-averaged diameters in the core regions of both laser-treated groups showed an increase from 3 to 6 weeks, while the sham group did not demonstrate this pattern (Table 4). This may indicate maturation of the newly formed collagen in the early process of remodeling. The consistently larger mass-averaged diameter in the laser-treated than the control group implied that LLLT had facilitated the repairing tissues to resemble the ultrastructural morphology of the intact MCLs. This observation is more prominent when comparing the core fibril sizes between the laser-treated and sham group at the 6-week time point (Table 4). In comparing the two laser-treated groups (Table 4), although the facilitation of fibril size appeared to be site-dependent, both groups show a general trend of increase in size along both testing periods. Fibrils of

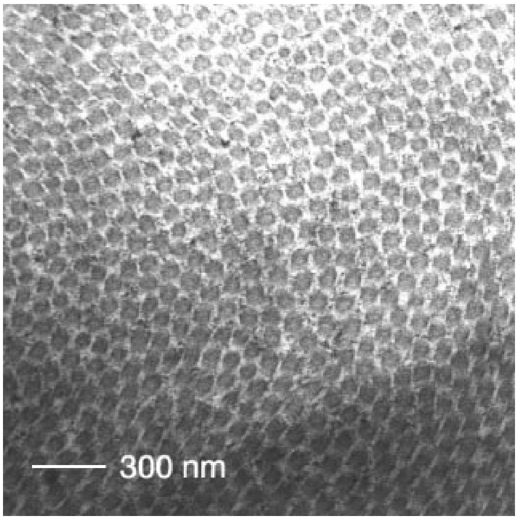

Fig. 1. Electron micrograph (×32,000) of the rat knee MCL middle peripheral region 3 weeks after laser therapy for 15 minutes.

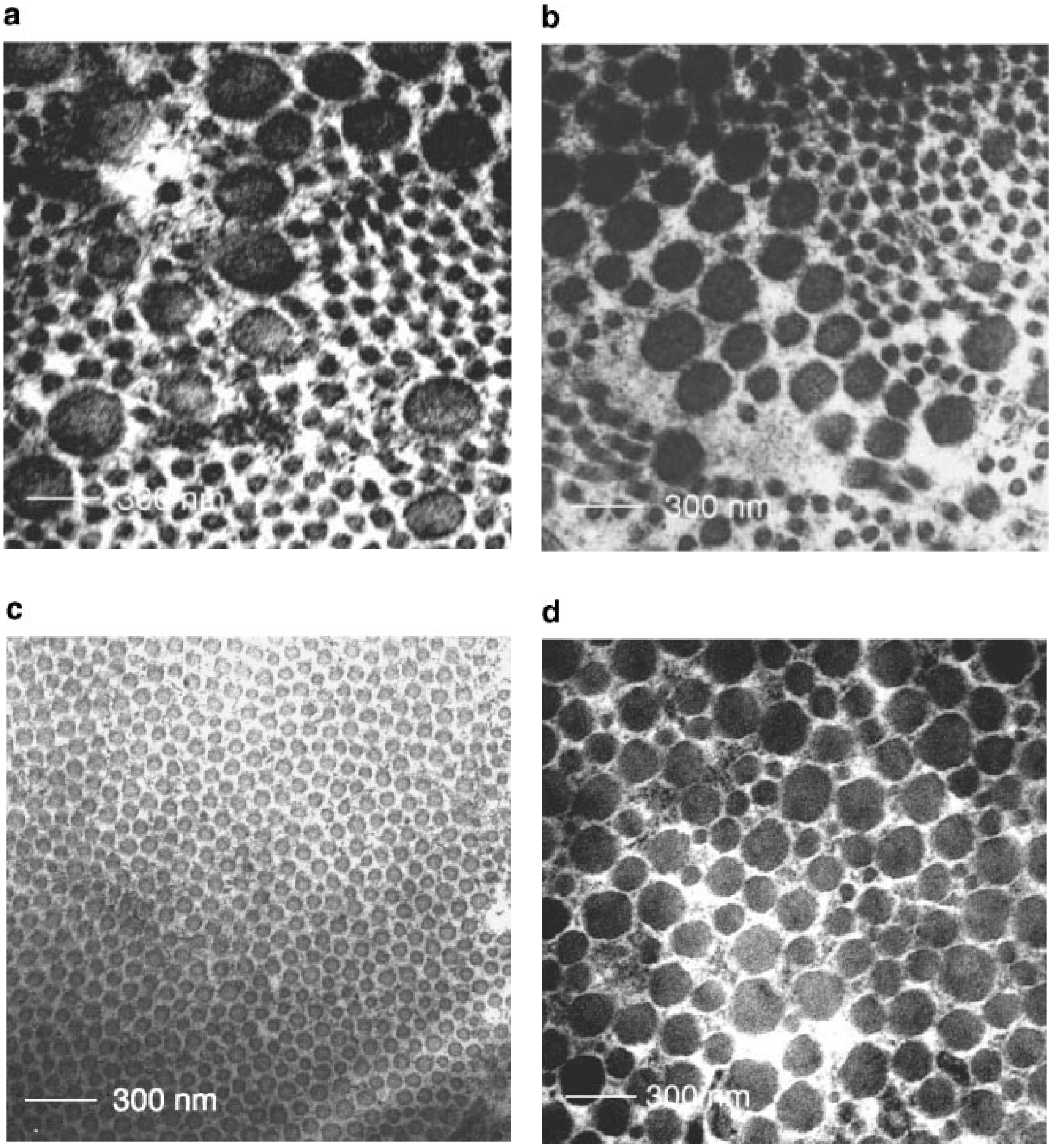

Fig. 2. **a**: Electron micrograph (×32,000) of the rat knee MCL middle core region 3 weeks after laser therapy for 15 minutes. **b**: Electron micrograph (×32,000) of the rat knee MCL middle core region 3 weeks after laser therapy for 7.5 minutes. **c**: Electron micrograph (×32,000) of the control rat knee MCL middle core region 3 weeks after surgery. **d**: Electron micrograph (×32,000) of the sham rat knee MCL middle core region 3 weeks after surgery.

the middle core region of group Laser7.5 was even larger in diameter than the sham group at 6 weeks post-injury (Table 4).

Results of the Kruskal–Wallis test and Mann–Whitney U-tests revealed significant difference between all groups of the same time frame and anatomical site ($P < 0.001$) (Table 5). The core fibrils of the sham group were about double in size of the other groups at 3 weeks. At 6 weeks post-injury, the values of both laser-treated groups were increased but still significantly smaller than the sham group (Table 5).

The patterns of mode obliquity were comparable among the different groups and sites. At 3 weeks, the values were between 0.62 and 0.84, whereas at 6 weeks, they were between 0.53 and 0.84. Table 6 shows the mode obliquity of all groups in the middle core and peripheral regions at the two testing times.

DISCUSSION

This study examined the ultrastructural collagen fibril morphology of healing MCL in rats after LLLT for either 15 or 7.5 minutes (dosages 63.2 and 31.6 J cm^{-2}), and

TABLE 2. Fibril Density of Experimental, Control, and Sham Groups at Different Sites and Time Post-Surgery

Parameter	Weeks after surgery	Testing site	Laser15	Laser7.5	Control	Sham
Fibril density (fibrils/μm^2)	3	Femoral core	85.17	81.71	133.99	31.63
		Femoral peripheral	109.08	106.88	133.13	112.84
		Middle core	134.69	78.43	154.30	29.87
		Middle peripheral	135.26	174.07	160.50	117.60
		Tibial core	87.80	157.80	138.02	30.15
		Tibial peripheral	132.55	146.84	139.22	130.29
Fibril density (fibrils/μm^2)	6	Femoral core	49.49	82.32	152.62	36.70
		Femoral peripheral	116.31	135.13	129.45	130.23
		Middle core	59.30	85.67	112.43	51.00
		Middle peripheral	113.23	133.01	148.53	108.12
		Tibial core	71.75	36.66	102.40	33.25
		Tibial peripheral	114.87	101.33	125.27	95.91

compared the collagen fibril profiles of the laser treated ligaments with the untreated control and sham operated groups. Our results revealed significant difference in collagen size among groups. Although the fibrils in both the peripheral and core sites of all groups were distributed in a unimodal manner, the actual fibril sizes were different. The mass-averaged diameters of both laser-treated (64.99–186.29 nm) and sham (64.74–204.34 nm) groups were larger than the controls (58.66–85.89 nm) at both 3 and 6 weeks post-surgery. Despite the fact that both laser-treated groups had similar mode diameters as the control group, the distributions were more heterogeneous in the core regions such that there were more large-size fibrils. These skewed distributions of collagen fibrils could imply that the laser treated MCLs were "remodeling" at a faster rate than the controls.

Our present findings have implications to the understanding of the mechanism underlying the therapeutic actions of LLLT on soft tissue healing. Enwemeka et al. [12] reported a trend of increase collagen fibril size with increasing laser dosages from 1 to 5 mJ cm^{-2} for Achilles tendon repair in rabbits. However, the dosages used in that study could be too low to show a significant effect. In the present study, we used much higher dosages of 31.6 and 63.2 J cm^{-2}, the laser-treated fibrils are found to be significantly larger than the untreated controls. Despite the fact that we cannot directly compare this study with the previous report [12], the findings may imply that the production and remodeling of collagen fibrils is dependent on the dosage of the laser treatment. Further studies are warranted to verify the relationships between collagen fibril profile and dosage of laser treatment.

Concerning the dosage of laser therapy, our findings showed that by increasing from 31.6 to 63.2 J cm^{-2}, no accompanying increase in fibril size was seen among different sites (Table 4). At a dose of 31.6 J cm^{-2} in group Laser7.5, the middle core fibril diameter of this group was even larger than the sham group at 6 weeks post-injury (Table 4). This could imply that there is an optimum dosage of laser therapy at which the collagen remodeling process is most enhanced.

The GaAlAs laser is categorized as a LLLT, the whole irradiated area should have been exposed to uniform photostimulation by the laser beam [28]. Since the rat MCL has a width of <0.2 cm, and the transected surgical wound was very small, the whole injured area was fully covered and uniformly irradiated by a single laser beam of area 0.125 cm^2 (diameter ~ 0.4 cm). In the present findings,

TABLE 3. Percentage Area Covered by Fibrils of Experimental, Control, and Sham Groups at Different Sites and Time Post-Surgery

Parameter	Weeks after surgery	Testing site	Laser15	Laser7.5	Control	Sham
%Area covered by fibrils	3	Femoral core	51.53	54.37	47.46	71.64
		Femoral peripheral	45.82	59.78	42.80	48.34
		Middle core	51.35	45.39	44.41	58.44
		Middle peripheral	48.44	49.80	42.63	49.55
		Tibial core	49.95	54.15	43.68	62.66
		Tibial peripheral	44.43	47.29	43.66	41.18
%Area covered by fibrils	6	Femoral core	47.26	48.20	52.59	60.44
		Femoral peripheral	46.61	50.96	52.84	52.48
		Middle core	45.55	53.83	50.24	54.80
		Middle peripheral	44.32	45.77	53.94	56.71
		Tibial core	51.24	56.97	47.82	54.41
		Tibial peripheral	42.55	51.22	47.99	36.92

TABLE 4. Mass-Averaged Diameter of Experimental, Control, and Sham Groups at Different Sites and Time Post-Surgery

Parameter	Weeks after surgery	Testing site	Laser15	Laser7.5	Control	Sham
Mass-averaged diameter (nm)	3	Femoral core	110.79	106.37	73.15	204.34
		Femoral peripheral	78.57	97.42	66.72	81.16
		Middle core	87.95	125.31	60.29	176.49
		Middle peripheral	75.81	64.99	58.66	73.58
		Tibial core	124.48	70.14	63.81	182.18
		Tibial peripheral	74.76	70.29	59.37	64.74
Mass-averaged diameter (nm)	6	Femoral core	153.07	119.23	72.14	192.78
		Femoral peripheral	89.19	79.02	76.29	77.33
		Middle core	123.24	186.29	80.89	152.99
		Middle peripheral	92.63	72.88	69.05	89.13
		Tibial core	154.91	177.09	85.89	177.41
		Tibial peripheral	81.77	93.18	70.50	75.98

the core fibrils remodeled to a large extent than the peripheral fibrils in the laser-treated groups. This could be due to the unique ultrastructural morphological characteristics in different anatomical sites of the rat MCL as demonstrated in normal specimens [24].

Parry et al. [21,22] reported positive relationships between collagen fibril size and strength in various tissues of different animals. Ng [29] studied the collagen size in repairing anterior cruciate ligament in goat and found that the collagen fibrils had gradually increased in size over time and there was a concomitant increase in breaking strength of the ligament. Based on these reports [21,22,29], our present findings can provide some hints on why biomechanical strength of repairing MCLs is improved with laser treatment [8]. However, these effects may only appear in the core MCL regions, as we found that the peripheral fibrils are less different in size among groups (Table 4).

In a study by Reddy et al. [11] on the effects of laser on Achilles tendon healing, no difference was found in the concentrations of hydroxypyridinium (HP) crosslinks between the laser treated tendons and controls. Since HP crosslink density correlates with tissue strength [29], if the improved biomechanical strength of the tissues treated with laser is not due to an increased HP crosslink density, it may have improved the "quality" of the collagen molecules per se rather than the inter-molecular forces. This is in line with our present findings that the collagen fibril size was increased with LLLT.

Obliquity refers to the fibril alignments relative to the cutting plane, which has only been briefly reported in the literature [16]. In the present study, the obliquity was generally comparable among all groups. Comparing to our previous findings on normal rat MCLs [24], the present values are lower. This could be due to the healing MCLs were within the initial fibril remodeling phase [25,30]. However, besides the obliquity index, we believe the pattern of fibril alignments should also be considered. If a sample had low obliquity index but the fibrils were all in-

TABLE 5. Mean Diameter and Standard Deviation of Experimental, Control, and Sham Groups at Different Sites and Time Post-Surgery

Parameter*	Weeks after surgery	Testing site	Laser15	Laser7.5	Control	Sham
Mean diameter and standard deviation (nm)	3	Femoral core	76.18 ± 33.83	82.59 ± 29.49	60.97 ± 17.32	144.19 ± 70.82
		Femoral peripheral	66.01 ± 19.04	76.18 ± 25.06	57.90 ± 14.80	68.20 ± 18.50
		Middle core	60.64 ± 23.94	70.83 ± 40.32	55.52 ± 11.55	140.06 ± 55.56
		Middle peripheral	61.33 ± 19.62	55.34 ± 15.41	52.36 ± 12.44	67.99 ± 13.98
		Tibial core	71.06 ± 38.84	61.39 ± 15.70	57.48 ± 13.41	147.00 ± 54.96
		Tibial peripheral	59.19 ± 19.61	57.19 ± 17.15	53.70 ± 12.14	58.67 ± 13.13
Mean diameter and standard deviation (nm)	6	Femoral core	94.78 ± 48.66	74.58 ± 34.64	60.15 ± 17.58	124.10 ± 64.18
		Femoral peripheral	63.27 ± 25.24	61.55 ± 20.52	64.73 ± 19.03	65.40 ± 18.58
		Middle core	85.29 ± 38.79	68.59 ± 51.70	68.28 ± 19.43	98.02 ± 53.46
		+Middle peripheral	59.83 ± 26.04	60.21 ± 18.08	61.52 ± 14.80	71.43 ± 23.82
		Tibial core	78.32 ± 45.06	121.67 ± 60.19	70.57 ± 21.69	127.88 ± 56.14
		Tibial peripheral	60.82 ± 22.09	72.32 ± 25.80	62.07 ± 16.02	64.63 ± 17.72

*Statistical differences were found in each pairwise comparison between groups in each testing site and time ($P < 0.001$).

TABLE 6. Mode Obliquity of Experimental, Control, and Sham Groups in the Middle Core and Peripheral Regions at two Periods Post-Surgery

Parameter	Weeks after surgery	Testing site	Laser15	Laser7.5	Control	Sham
Mode obliquity	3	Middle core	0.74	0.62	0.84	0.69
		Middle peripheral	0.74	0.81	0.84	0.84
Mode obliquity	6	Middle core	0.69	0.84	0.81	0.64
		Middle peripheral	0.81	0.67	0.84	0.81

clined to the same direction, it might be functionally better than those with higher obliquity index but diverse inclination patterns.

In order to ensure a valid comparison among groups, variations both within and among samples were kept to a minimum throughout the preparation of the tissues and collection of data. This was achieved by standardizing the procedures in tissue processing and cutting; sampling; normalizing the electron beam; and calibrating the electron microscope during image taking. Furthermore, "equality of artifact" both within and between samples was assumed. Some degrees of variability being measured were acknowledged as a result of artifact variation. With this factor being identified clearly, it could be assumed that both qualitative and quantitative comparisons of data were justified [16]. Overall, the data in our sham operated MCLs agreed with previous reports of the same tissue [14], as well as other connective tissues such as anterior cruciate ligament in goat [29] and MCL in rabbit [15,16]. In our previous study [24], we found that different anatomical sites of the MCL have different distributions. Therefore comparisons between treatment groups were done on separate anatomical sites in this study.

We used a "systemic random sampling" method in this study [21,22,25]. This method has been adopted extensively in quantitative studies, which was regarded to be less biased and can provide a more genuine representation of the overall estimate of the population [21,22,25]. However, Frank et al. [16] commented that in order to avoid artifacts such as the pericellular areas which contained a high proportion of small diameter fibrils, or fissures resulted from tissue processing and dehydration procedures, a deliberate biased sampling would be more preferable. In addition, Parry et al. [22] stated that in order to obtain a true representation of the collagen fibril population, between 500 and 5,000 collagen fibrils per ligament should be analyzed according to the degree of heterogeneity. In this study, we analyzed a total of 399,817 fibrils, which means an average of over 2,000 fibrils for each of the 6 sites per ligament. This sampling method and large number of fibrils being analyzed would have assured validity of our data.

In view that the healing process of connective tissues comprises inflammation, repair, and remodeling [25,30] and each phase has its unique biological properties, we were interested in the active repair and early remodeling phases because the strength of the repairing tissue increases in these two stages. Therefore two time intervals of 3 and 6 weeks were examined. However, Lyons et al. [31] and Reddy et al. [11] reported that the effects of laser on soft tissues could happen as early as 2 weeks post-injury. We suggest future studies to include a time frame of 2 weeks or 10 days post-injury so that the early effects of laser could be examined.

CONCLUSION

We conclude that a low energy GaAlAs laser therapy can significantly restore collagen fibril sizes at 3 and 6 weeks after complete transection injury of the MCL in rats.

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