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Abstract. The effects of laser polarization on the efficacy of near-infrared low-level laser therapy for spinal cord injury (SCI) are presented. Rat spinal cords were injured with a weight-drop device, and the lesion sites were directly irradiated with a linearly polarized 808-nm diode laser positioned either perpendicular or parallel to the spine immediately after the injury and daily for five consecutive days. Functional recovery was assessed daily by an open-field test. Regardless of the polarization direction, functional scores of SCI rats that were treated with the 808-nm laser irradiation were significantly higher than those of SCI alone group (Group 1) from day 5 after injury. The locomotive function of SCI rats irradiated parallel to the spinal column (Group 3) was significantly improved from day 10 after injury, compared to SCI rats treated with the linear polarization perpendicular to the spinal column (Group 2). There were no significant differences in ATP contents in the injured tissue among the three groups. We speculate that the higher efficacy with parallel irradiation is attributable to the deeper light penetration into tissue with anisotropic scattering. [DOI: 10.1117/1.JBO.18.9.098002]

Keywords: low-level laser therapy; photobiomodulation; polarization; spinal cord injury; functional evaluation.

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1 Introduction

In spinal cord injury (SCI), complete or partial loss of autonomic, sensory, and motor functions is caused by interruption of neural signal conduction along the axonal tracts. There is generally poor recovery of these functions because of the difficulty of tissue regeneration in the central nervous system. Thus, SCI patients are left with serious residual disabilities, such as paralysis, respiratory difficulty, chronic pain, urinary problems, and neurologic decline, leading to considerable decrease in quality of life. Various strategies have been examined for repair of SCI in animal models, including blockage of the endogenous growth inhibitory factors,[1,2] infusion of neurotrophic factors,[3,4] and transplantation of growth promoting cells.[5–7] However, no effective treatment for SCI has yet been established.

Low-level laser therapy (LLLT) is a promising approach to treat SCI. LLLT has been clinically applied to the treatment of rheumatoid arthritis and periodontal disease, pain management, and healing of wounds and burns.[8–10] LLLT is also currently used for the treatment of various neurological diseases such as stroke, neurodegenerative diseases, and brain injury.[11–16] Several studies have shown that near-infrared LLLT has the potential to be an effective noninvasive therapy for SCI.[17–20] Rochkind et al. demonstrated that transplantation of embryonal spinal cord nerve cells followed by 780-nm laser irradiation enhanced axonal sprouting and spinal cord repair in a completely transected rat SCI model.[17] In two different rat models of hemisection SCI and contusion SCI, Anders et al. transcutaneously applied an 810-nm laser, which penetrated to the depth of the injured spinal cord and promoted axonal regeneration and functional recovery.[18,19] Their study demonstrated that near-infrared laser irradiation significantly suppressed immune cell activation and cytokine/chemokine expression, suggesting that a decrease in the inflammatory response is one of the recovery mechanisms in LLLT for spinal cord repair.

The detailed mechanisms of LLLT are still under investigation. However, the therapeutic efficacy relies fundamentally on the initial photochemical event, i.e., absorption of photons by photoacceptors or chromophores such as cytochrome *c* oxidase in the tissue.[21,22] Karu et al. showed in an *in vitro* study that the basic processes of LLLT occurring in HeLa cells were light absorption and photochemistry but that the incident characteristics of photons, such as degree of light polarization, did not affect the biological reactions in LLLT.[23] However, scattering of photons *in vivo* depends on the microstructure of tissue, and light propagation into biological tissue would therefore change the healing property. For instance, Ribeiro et al. investigated the repair of skin burns in rats with a linearly polarized He–Ne laser beam, which was parallel or perpendicular to the direction of the spinal column, at the same laser dose.[24,25] Their results showed that the healing process was dependent on the polarization orientation; lesions irradiated with parallel

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polarization were completely repaired 17 days after wound creation, while those with perpendicularly polarized irradiation showed a moderate degree of healing in the same period. They attributed these results to the fact that the parallel polarization was aligned with the predominant orientation of collagen fibers in the dermis, which was confirmed by histological analysis. This alignment would reduce photon scattering and thus increase optical penetration depth in the tissue, leading to the acceleration and improvement of cutaneous wound repair.[24]

It is widely known that photon scattering by aligned cylindrical structures, such as myofibrils, axons, and collagen fibers, results in anisotropic light reflection and propagation in the tissue.[26–30] These characteristics are often used for diagnosis of the tissue abnormalities and for mapping of specific structures in the tissue.[31–33] Since the spinal cord has a fibrous structure, photon migration should be affected by polarization of incident light in the tissue. However, the effect of polarization on efficacy of LLLT for SCI has not been elucidated. In the present study, we examined the effect of relative orientation of laser polarization on efficacy of near-infrared LLLT for contused spinal cords in rats.

2 Materials and Methods

2.1 *Spinal Cord Injury Model*

The protocol used in this study was approved by the Committee on Ethics of Animal Experiments in the National Defense Medical College. We used female Sprague-Dawley rats (Japan SLC Inc., Shizuoka, Japan) weighing 180 to 270 g. Before the operation, they were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg animal weight). During all of the experiments using anesthesia, body temperature was monitored with a rectal probe and maintained at 37.0 to 37.5°C. The lamina of the tenth thoracic vertebra was surgically removed, and the spinal cord was exposed. A New York University weight-drop device was used to make a severe spinal cord contusion.[34–36] A 10-g metal rod with a flat circular impact surface 2.5 mm in diameter was dropped from a height of 25 mm onto the exposed spinal cord.

2.2 *Laser Irradiation*

Immediately after making a contusion in the spinal cord, the lesion site was directly irradiated with an 808-nm diode laser beam (B&W Tek Inc., Newark, Delaware) that was transmitted through a polarizer (SPFN-30C-26, Sigma Koki Co., Ltd., Tokyo, Japan). The polarizer was held with a rotatable holder to change the direction of incident polarization onto the tissue. The incised skin was closed with sutures after laser irradiation. Exposure of the spinal cord, irradiation, and suturing were repeated daily for the following five consecutive days. The control animals (Group 1) received a weight-drop injury and the lesion was exposed daily but not irradiated with the laser beam. The injured tissue of Group 2 rats was irradiated with a linearly polarized laser perpendicular to the spinal direction (hereafter called perpendicular polarization) and that of Group 3 rats was treated with parallel-aligned polarization (hereafter called parallel polarization). The laser power measured at the injured spinal cord surface was 25 mW, with a spot diameter of 20 mm giving a power density of 8 mW/cm^2. Using an irradiation duration of 20 min, the light fluence per day was 9.6 J/cm^2.

2.3 *Functional Evaluation*

The motor function of hind limbs was evaluated by open-field testing and scored on the basis of the Basso-Beattie-Bresnahan (BBB) scale[35,36] ($n = 12$ in each group); a score of 0 means no spontaneous movement, while a score of 21 indicates normal locomotion. Assessment of the animals was performed before laminectomy and 1, 2, 3, 5, 7, 10, 14, and 21 days after injury. The open field consisted of a squared arena (45 cm × 90 cm) with 20-cm-high walls. All rats received manual bladder expression before the open-field test to eliminate possible behavior differences due to bladder fullness.

2.4 *Histological Analysis*

After spinal injury, glial cells are intrinsically activated with enlarged somas and intensive expression of intermediate filament proteins over time as the inflammatory response.[37,38] The activated astrocytes compose a glial scar, forming a cystic cavity in the region surrounded by the scar.[37,38] This neurodegenerative nature leads to a progressive increase in the size of the cavitation area.[39] Thus, a cavity is closely connected with inflammation. Since the locomotor recovery is critically correlated with the percentage of remaining normal nerve fibers in spinal tissue,[40,41] suppression of the excessive inflammatory responses and progressive increase in the lesion sizes is necessary. Thus, we evaluated cavity area as the most important histopathological outcome.

Under systemic anesthesia, rats were euthanized 21 days after injury by transcardial perfusion with 150 mL physiological saline followed by further perfusion with 200 mL 4% paraformaldehyde in physiological saline. Segments of the spinal cords centering on the injury were removed and postfixed in the same fixative overnight. The tissues were then frozen in an optimal cutting temperature compound (Sakura Finetek USA Inc., Torrance, California) and sectioned to 10-μm-thick slices with a cryostat microtome. For histological images (HE staining) of the longitudinal sections at the lesion epicenter, cavity areas were manually outlined and quantified by image analysis using Adobe Photoshop 7.0 imaging software (Adobe Systems, San Jose, California) ($n = 9$ in each group).

2.5 *ATP Content Measurement*

Increase in ATP synthesis is one of the important indicators for evaluating the effect of LLLT on enhancement of mitochondrial function.[22] Immediately after near-infrared laser irradiation, we harvested traumatized spinal tissues (length, ~1 cm) and measured ATP contents in the tissues with an ATP assay kit (TA100, Toyo Ink., Tokyo, Japan) according to the manufacturer's instructions ($n = 6$ in each group). Intact spinal tissues (normal) and injured tissues after laminectomy without laser irradiation (Group 1) were also harvested and analyzed for comparison. Spinal tissue was homogenized in 1 mL of 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid buffer, and the solution was centrifuged at 15000 rpm for 15 min at 4°C to pellet insoluble materials, followed by addition of the ATP-extraction buffer provided in the kit to a portion of the lysis solution. After shaking and incubating for 30 min at room temperature, the supernatant was mixed with luciferin, and then luminescence of an aliquot was measured with a luminometer (LB955, Berthold Technologies, Bad Wildbad, Germany). The concentration of ATP was calculated using the ATP standard curve

and expressed as nmol per mg protein. Protein concentrations in all spinal samples were determined using a protein assay system (500-0112, BioRad, Richmond, California).

2.6 *Distribution of Light Transmitted Through Spinal Tissue*

To compare penetrations of light with different polarization directions through the spinal cord, light transmitted through excised spinal tissue was imaged with a CCD camera (XC-7500, Sony Corp., Tokyo, Japan). The experimental setup is schematically shown in Fig. 1. A fresh spinal column removed from an uninjured rat (diameter, ~5 mm; length, 12 mm) was placed on a black plastic sheet (thickness, 0.7 mm) with a rectangular hole (3 mm × 9 mm) through which polarized laser light was directed onto the bottom surface of the spinal column ($n = 2$). The transmitted light was detected from the top. The polarization direction was changed by rotating a polarizer that was placed between the fiber output end and the spinal tissue; 0 and 180 deg means the incident direction of the linear polarized laser was parallel to the spinal column, while 90 deg indicates the polarization direction was perpendicular to the spinal column. The laser power measured at the bottom surface of the spinal cord was 25 mW. The transmitted light was quantified by calculating white-colored pixels in the regions of interest (ROIs, 3 mm × 9 mm), corresponding to the size of a rectangular hole in the plastic sheet.

2.7 *Statistical Analysis*

The results of functional evaluation were compared between the groups using two-way repeated analysis of variance (ANOVA) with Tukey's *post hoc* test. Statistical analysis for the results of cavity area and ATP content measurement was performed using one-way factorial ANOVA followed by Tukey's *post hoc* test. A value of $P < 0.05$ was regarded as statistically significant.

3 Results

3.1 *Functional Recovery*

Figure 2 shows the BBB scores for rats in the three groups as a function of time after injury. Regardless of the polarization direction, the BBB scores of the rats receiving 808-nm laser irradiation (Groups 2 and 3) were significantly higher than those of

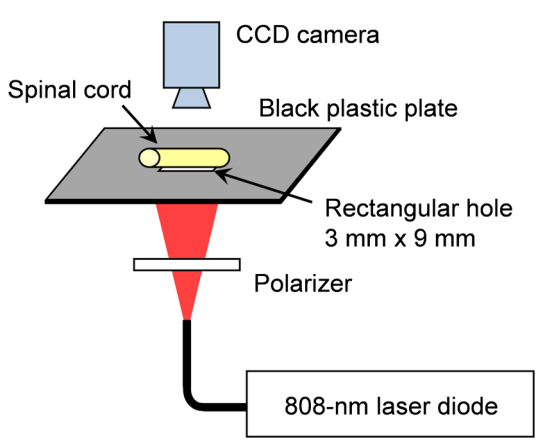

Fig. 1 Experimental setup for measurement of light transmitted through an excised spinal tissue.

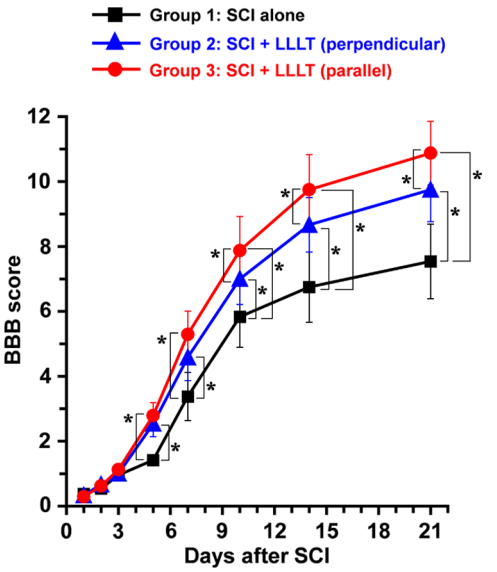

Fig. 2 Locomotive scores based on an open-field test as a function of days after SCI. Values are expressed as means + standard error of the mean (SEM) ($n = 12$). Asterisks indicate significant differences ($P < 0.05$).

the SCI alone group (Group 1) from five days after injury (Group 1 versus Group 2, $P = 0.003$ at day 5; $P = 0.002$ at days 7 and 10; $P = 0.000$ at days 14 and 21, Group 1 versus Group 3, $P = 0.000$ at days 5, 7, 10, 14, and 21). In addition, BBB scores of the rats irradiated with parallel polarization (Group 3) were significantly higher than those of the rats treated with perpendicular polarization (Group 2) from 10 days after SCI ($P = 0.029$ at day 10; $P = 0.005$ at day 14; $P = 0.003$ at day 21). The averaged BBB scores at three weeks post-SCI were 7.5 ± 1.2 for Group 1, 9.8 ± 1.0 for Group 2, and 10.9 ± 1.0 for Group 3.

3.2 *Histologic Evaluation*

Figure 3 shows histological images (HE staining) of longitudinal sections of the spinal cords of rats in all groups at three weeks after injury. Formation of a cavity was observed in the spinal cord tissue of all rats. Figure 4 shows the results of quantitative analysis of the cavity areas in injured spinal cords on the basis of histological images. Cavity areas for the rats in Groups 2 and 3 were significantly smaller than those for the rats in Group 1; $P = 0.050$ between Groups 1 and 2 and $P = 0.006$ between Groups 1 and 3, while $P = 0.639$ between Groups 1 and 2. These results show that irradiation with both the parallel and perpendicular polarized laser can lead to reduced formation of glial scar and cavity. However, there was no significant difference in cavity area between Groups 2 and 3.

3.3 *ATP Content*

Figure 5 shows the ATP content in the spinal cords of rats in Groups 1, 2, and 3, where the value for normal rats is also shown for comparison. In the normal rats, baseline ATP content in the spinal tissues was 0.13 ± 0.02 nmol per mg protein, compared with 0.07 ± 0.02 nmol per mg protein in the untreated SCI rats (Group 1). The ATP contents in rats of Groups 2 and 3 were 0.08 ± 0.02 and 0.09 ± 0.02 nmol per protein, respectively. However, there was no significant difference in ATP content between Groups 1, 2, and 3, indicating that

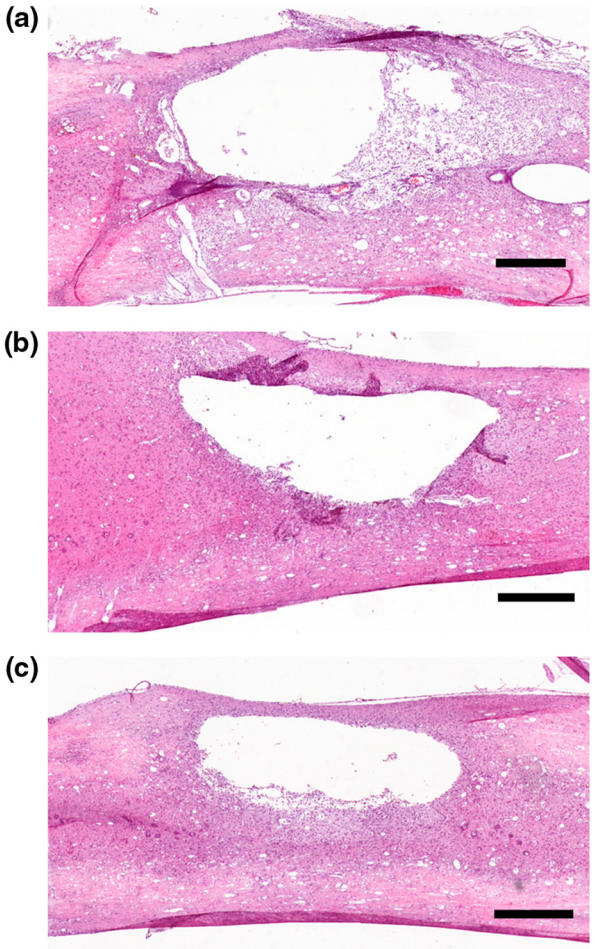

Fig. 3 Histological images (HE staining) of longitudinal sections of injured spinal cords at three weeks after injury: (a) Rat with SCI alone (Group 1). (b) SCI rat treated with perpendicularly polarized laser to the spinal column (Group 2). (c) SCI rat treated with parallel polarization (Group 3). Scale bars indicate 200 μm.

ATP synthesis immediately after LLLT was not associated with improved motor function by near-infrared light either with parallel or perpendicular polarization.

3.4 *Light Transmitted Through the Spinal Cord*

Figure 6 shows the distributions of light transmitted through an excised spinal cord under two incident polarization conditions: (a) perpendicular and (b) parallel to the spinal direction. Figure 6(c) shows the amount of transmitted light from the ROI as a function of incident laser polarization direction. The amount of transmitted light for parallel polarization was ~1.8-fold higher than that for perpendicular polarization, indicating that light with parallel polarization penetrated deeper in the spinal tissue than did light with perpendicular polarization.

4 Discussion

The current study has shown that locomotive scores of SCI rats with 808-nm laser treatment were significantly higher than those of SCI rats without light irradiation from day 5 after injury onward regardless of the incident polarization direction (Fig. 2). Anders et al. demonstrated that transcutaneous application of 810-nm nonpolarized laser significantly promoted axonal

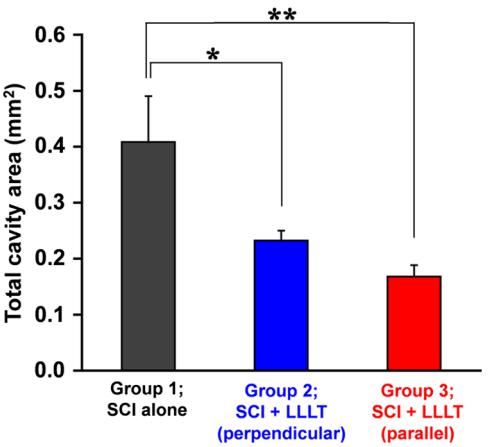

Fig. 4 Results of quantitative analysis of injury cavity areas in spinal cords on the basis of histological images. Values are expressed as means ± SEM ($n = 9$ in each group). *$P < 0.05$, **$P < 0.01$.

regrowth at six weeks postinjury in a rat hemisection SCI model[18] and functional recovery at three weeks after injury in a rat contused SCI model.[19] There are differences in the time course of treatment efficacy between our study and their studies. In their experiments, the incident laser power and daily dosage at the skin surface overlying the lesion site were 150 mW and 1589 J/cm^2 (irradiation duration, 2997 s), respectively, 6% of which (9 mW and 95 J/cm^2) penetrated to the spinal cord depth. The irradiation was applied daily for 14 consecutive days after SCI.[18,19] They concluded that the improved axonal regeneration was caused by inhibiting inflammatory cell activity due to laser irradiation at high dosage per day (>10 J/cm^2). In our study, on the other hand, a linearly polarized laser was directly applied to the exposed spinal cord lesion immediately after trauma and then daily for the following five days at the fluence of 9.6 J/cm^2 (power, 25 mW; irradiation duration, 1200 s). The therapeutic effects of near-infrared laser irradiation have been reported to be dependent on dosage, being associated with production of anti-apoptotic, pro-proliferative, antioxidant, and angiogenic factors.[22,42–44] Although further study is needed to clarify the therapeutic mechanisms, as well as the optimum irradiation conditions for treating SCI, a different mechanism might work for treatment under the laser irradiation conditions in the present study.

Locomotor function of SCI rats treated with parallel polarization (Group 3) was significantly improved when compared

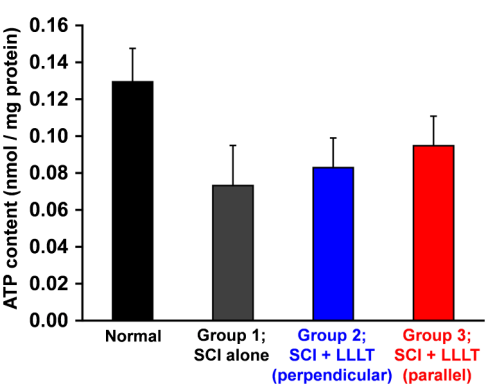

Fig. 5 ATP contents in normal and injured spinal cords (Groups 1, 2, and 3). Results are expressed as means + SEM ($n = 6$ in each group).

with that irradiated with perpendicular polarization (Group 2) from day 10 after trauma (Fig. 2). To investigate the reason for the polarization-dependent treatment efficacy, we evaluated the cavity area 21 days after injury (Figs. 3 and 4), ATP content immediately after SCI (Fig. 5), and light penetration in the spinal tissue (Fig. 6). There was no statistically significant difference either in cavity area or in ATP content between the two polarization groups. On the other hand, the polarization dependence of light transmission through tissue was remarkable; the amount of light transmitted through the spinal cord with perpendicular polarization was ~40% less than that with parallel polarization [Fig. 6(c)]. Thus, we speculate that the higher treatment efficacy with parallel polarization is attributable to the more efficient light propagation through tissue, which is consistent with the results reported by Hebeda et al.[26] The penetration depth of red light (wavelength, 632.8 nm) with polarization parallel to white matter tracts in a fresh bovine brain was significantly greater than that of red light polarized perpendicular to myelinated fiber tracts; the effective attenuation coefficients of light (μ_{eff}) were 0.47 ± 0.06 mm^{-1} with parallel polarization and 0.63 ± 0.13 mm^{-1} with perpendicular direction ($P < 0.05$).[26] It is known that locomotor recovery after SCI is highly correlated with the volume of remaining normal nerve fibers in spinal tissue.[40,41] In the present study, LLLT with parallel polarization would have provided more efficient protection of neural cells from apoptosis or necrosis in deep-located anterior horns than LLLT with perpendicular polarization. Further investigation is needed to elucidate the detailed physiological contributions of linearly polarized light, but the present study clearly demonstrated the importance of light polarization in LLLT for SCI.

Moreover, Silva et al. recently reported that when the rat tendon was irradiated with linearly polarized 632.8-nm laser aligned in parallel with the tendon long axis, the tissue showed higher birefringence and greater nonsusceptibility when compared with those of the nonirradiated tissue. They attributed this to the changes in molecular order and aggregation states of the collagen fibers.[45] With linearly polarized laser, the tendon was irradiated once in their work, while the spinal cord was irradiated daily for five consecutive days in our work. Similar alteration of the fibrotic structure, which affects direction of photon scattering, could take place in the spinal cord examined in this study.

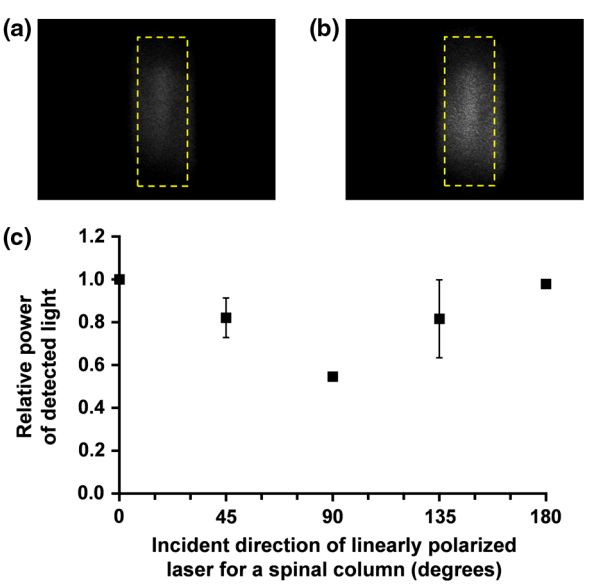

Fig. 6 Images of laser light transmitted through excised spinal tissue: directions of incident linearly polarized laser were (a) perpendicular and (b) parallel to the spinal column. (c) Transmitted light distribution in ROIs [areas indicated by a broken line in (a) and (b)] as a function of the direction of incident linearly polarized laser with respect to spinal orientation. Values are expressed as means ± SD ($n = 2$).

Open surgery for direct laser irradiation to the spinal cord is invasive, which is not practical in clinical situations. However, there seems to be a chance for direct irradiation during surgical exposure for spinal decompression and endoscopic discectomy, for which laser delivery through an optical fiber should be useful. For fiber-based irradiation with a linearly polarized laser, a polarization filter can be placed at the laser output fiber end, or a polarization-maintaining fiber can also be used.

Although we observed a significantly higher treatment efficacy with parallel polarization than with perpendicular polarization, the difference is not large enough to indicate medical relevance, although our results would provide important data concerning effect of light polarization in LLLT for fibrotic tissue. It should also be noted that our data strongly support the efficacy of LLLT for SCI regardless of the polarization direction. In addition to transcutaneous LLLT,[18,19] LLLT based on direct laser irradiation can also provide a therapeutic option for SCI since open surgery is often performed for decompression in conventional SCI treatment strategy.

5 Conclusion

We investigated the effects of polarization on efficacy of 808-nm LLLT for contusion SCI in rats. Rats treated with light for which polarization was parallel to the spinal direction showed significantly faster recovery of locomotor function than did rats treated with perpendicular polarization. We speculate that this is attributable to deeper photon penetration through spinal tissue with parallel polarization than with perpendicular polarization.

Acknowledgments

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