Helical Conformation Endows Poly-L-Lactic Acid Fibers with a Piezoelectric Charge under Tensile Stress

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ABSTRACT. Poly-L-lactic acid (PLLA) has been clinically used as a bioabsorbable material and attains a piezoelectric charge upon molecular orientation by the application of a shear force to the C-axis of the crystal line region. Previous studies showed that implanted drawn PLLA films or rods accelerate the ossification due to piezoelectric effect. In this study, we originally designed helically-twisted PLLA fiber to produce piezoelectricity in bioabsorbable suture upon tensile stress. The piezoelectricity of the helical PLLA fibers was evaluated using a lock-in amplifier system *in vitro*. The ossification induced by helical PLLA fibers was examined by implanting them in the rat patellar ligament supporting a physiological tensile load. We observed that 57° and 45° twisted PLLA fibers generated a higher piezoelectric potential than did 27° twisted fibers. The animal experiment showed that the formation of osseous tissue around helical PLLA fibers was more significant than around non-helical control fibers at 4 weeks after their implantation. These results suggest that helical PLLA fiber may be useful for the surgical suture or artificial ligament, which connects to the bone.

KEY WORDS: animal model, helical conformation, ossification, piezoelectricity, poly-L-lactic acid.

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Ligament injury, such as anterior cruciate ligament rupture, is a common cause of lameness in small animal practice [6, 15]. Various types of artificial ligaments including bioabsorbable material have been studied to reconstruct the injured ligament [3, 27, 31]. It has been suggested that early osteointegration of the grafted artificial ligament to the bone is important to improve the ligament function and durability [39].

Poly-L-lactic acid (PLLA) is well known as a bioabsorbable material and has been clinically used as bioabsorbable mini-plate, screw and pin [2, 13, 14, 30, 33]. On the other hand, PLLA is known as a piezoelectric material. Fukada reported that an external mechanical stress to a drawn PLLA film gives rise to electrical polarization [10], and this piezoelectric effect is ascribed to the change in the microscopic orientation of crystals with a fundamentally asymmetrical structure [5, 10, 22, 23]. Animal experiments showed that the piezoelectricity of drawn PLLA films and rods accelerated callus formation on bone [10, 22, 35]. Shimono *et al.* prepared three types of PLLA piezoelectric films with dif-

ferent oriented directions (0°, 45° or 90° to the drawing direction) and implanted them on the periosteum of the rabbit tibia [35]. These drawn PLLA films promoted ossification to a greater extent than undrawn PLLA films, and the effect of drawn PLLA films in promoting ossification was greatest when a shearing stress was applied at 45° to the axis of orientation. Ikada *et al.* prepared drawn PLLA rods with different draw ratios and implanted these rods intramedullary in the osteotomized tibia of cats [22]. Fracture healing was clearly promoted with increased callus formation as the draw ratio of the PLLA rod increased, whereas the undrawn PLLA rods had no effect on callus formation. These reports suggest the possibility that PLLA fibers can be used as bioabsorbable artificial ligaments or surgical sutures that have the potential to improve the osteointegration [17, 18, 20, 21, 34]. However, there is no direct evidence of the piezoelectric effect. As animal experiments have many factors that affect the bone formation, it is difficult to produce the reliable evidence and to elucidate mechanism. Moreover, piezoelectricity is rarely generated when a tensile force is applied to drawn PLLA fibers, because the piezoelectric effect is caused by the application of shear stress to the axis of the crystals. To address this issue, we designed PLLA fibers with a helical conformation to induce piezoelectricity under dynamic tensile stress. This study measured the piezoelectric potential of the helical PLLA fibers, and animal experiment was performed to show the clinical relevancy.

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Fig. 1. Scanning electron microscopic view of a helical poly-L-lactic acid (PLLA) fiber (A). Schematic representation of the apparatus to measure the piezoelectric potential (B).

MATERIALS AND METHODS

Measurement of piezoelectric potential: Helical PLLA fibers for *in vitro* examination were obtained by twisting a drawn PLLA tube (GUNZE LIMITED, Kyoto, Japan, 1.5 mm in diameter) at a rate of 1.5 radians per min in silicone oil (Shin-Etsu Silicone, Tokyo, Japan, KF-96) at 80°C. Immediately after twisting, the silicone oil was heated up to 110°C to crystallize the PLLA fibers. Six varieties of twist angle were fixed at $\pm 27^\circ$, $\pm 45^\circ$ and $\pm 57^\circ$ by monitoring the outer surface of the PLLA fiber (Fig. 1A). Helical PLLA fibers for *in vivo* testing were obtained by twisting a drawn PLLA fiber (GUNZE LIMITED 0.3 mm in diameter) in the same manner. An untwisted PLLA fiber used as a control was crystallized in the same manner.

The tension-induced piezoelectric potential of 42 samples, 6 samples of each twist angle, was measured between the inner and the outer surfaces of the PLLA fiber using a two-phase lock-in amplifier (NF Corporation, Yokohama, Japan, 5610B) and custom-designed tension device system (Fig. 1B). The platinum coating on the outer surface of PLLA fiber leveled-off the potential of the outer surface, whereas the mercury-filled internal cavity of the fiber leveled-off the potential of the inner surface. The electrical potential between the platinum coat and the mercury-filled cavity was measured. Tensile forces (2.4 N, 3.2 N, 4.0 N and 4.8 N) were cyclically applied to the PLLA fiber in the direction of the long axis by solenoid actuator via tension device. The solenoid actuator was driven by a sine-wave signal from the 2-phase lock-in amplifier at a frequency of 7 Hz. The piezoelectric potential synchronized to the sine-wave was detected using the two-phase lock-in amplifier.

Animal experiments: Animal experiments were carried out to examine the histological reaction (osteointegrative response) to the helical PLLA fibers, in accordance with the

Fig. 2. Schematic drawing (A) and picture (B) of the surgical procedure.

guidelines for animal experimentation at Kyoto University. All procedures were approved by the Animal Care and Use Committee of Kyoto University. Twelve Fischer 344 rats (male, 13 weeks old, 240–250 g) were anesthetized by intraperitoneal injection of xylazine (10 mg/kg body weight) and ketamine (90 mg/kg body weight). After the preparation, the skin on the knee joint was incised using the medial parapatellar approach. A 1-mm drill hole was longitudinally created in the patellar and tibial tuberosity, and a 1-mm width cut was made in the center of the patella ligament. The PLLA fibers were then tied to the distal end of the quadriceps femoris muscle and tibial tuberosity by passing them through the drill hole in the patella and tibia (Fig. 2). Twelve helical

PLLA fibers (300 μm diameter and 45° twist angle) were implanted in the left knee joint, whereas 12 non-helical PLLA fibers (control) were implanted in the right knee joint. No rats showed infection or loss in body weight during the study period. Six rats were euthanized at 2 weeks after surgery, and remaining 6 were euthanized at 4 weeks after surgery by inhalation of overdose of diethyl ether. The harvested knee joints were fixed in 4% paraformaldehyde at 4°C for 24 hr and then decalcified with 10% EDTA. Transversely sectioned proximal tibia was obtained from each specimen, and the formation of osseous tissue around the PLLA fibers was histologically examined using hematoxylin and eosin staining. The contact ratio of the osseous tissue around the PLLA fibers was calculated at a low magnification view by the circular arc length of the regenerated osseous tissue divided by the circumferential length of the PLLA fibers. The circular arc length and the circumference were measured using an image analysis software "NIH image". Results are presented as mean $\pm$ standard deviation. Statistical analysis was performed by Student's *t*-test. Differences were considered significant when $P < 0.05$.

RESULTS

Piezoelectric potential of helical PLLA: The relationship between the tensile force applied to the PLLA fibers and the generated piezoelectricity is shown in Fig. 3. The piezoelectric potential was in proportion to the tensile force for each twisting angle. The highest piezoelectric potential upon tension was observed in the PLLA fibers with a $\pm 57^\circ$ twist. The phases of the piezoelectrical peaks were observed at $+37.9^\circ$ and -130.8° for the clockwise-twisted fibers and at -107.4° and $+47.2^\circ$ for the counterclockwise-twisted fibers. No piezoelectric potential was detected in the control fibers.

Histological examinations: Numerous spindle-shaped mesenchymal cells were observed around the fibers in both groups at 2 weeks after the operation (Fig. 4). At a higher magnification, the cells were regularly and smoothly attached to the surface of the helical PLLA fibers (Fig. 4A), whereas the cells in the control group were irregularly shaped around the non-helical PLLA fibers (Fig. 4B). The implanted fibers were in contact with osseous tissue at $5.1 \pm 9.5\%$ of the circumference in the control group, whereas $22.2\% \pm 16.0\%$ of the circumference of the fibers was surrounded by osseous tissue in the helical-PLLA group ($P < 0.05$; Fig. 5).

Progressive bone formation was observed around the fibers at 4 weeks after the operation. About 50% of the helical-PLLA fiber surface was attached to the trabecular bone (Fig. 4C), whereas osteoblast lines of ossification were observed on the bone around the control fibers (Fig. 4D). Only $8.8 \pm 6.1\%$ of the circumference was surrounded by osseous tissue in the control group, whereas $51.8 \pm 32.2\%$ was surrounded in the helical-PLLA group ($P < 0.01$; Fig. 5).

DISCUSSION

To our knowledge, there are no reports on the developmental research of piezoelectricity-induced artificial

Fig. 3. Comparison of the piezoelectric potential derived from helical PLLA fibers with different degrees of twisting. Significant differences were observed between 27° and 45° twisted PLLA fibers, and between 27° and 57° twisted PLLA fibers (**: $P < 0.01$).

ligaments or suture materials. This is the first report, which demonstrates that piezoelectric potential was induced by the helical conformation on the PLLA fiber.

The piezoelectric strain constant (*d*) is given by the electric displacement (*D*) differentiated by the mechanical stress (*T*) or the mechanical strain (*S*) differentiated by the electric field (*E*) [10].

$$d = (\partial D / \partial T)_E = (\partial S / \partial E)_T \quad (1)$$

$$d_{14} = P_x / T_{yz} \quad (2)$$

$$d = (d_{14} / 2) \sin 2\theta \quad (3)$$

Although, it has been suggested that the piezoelectric effect on bone formation is difficult to prove, animal experiments are the only way to detect the clinical relevancy. Some clinical studies and animal experiments have suggested enhancement of ossification by electrical stimulation [1, 7, 11, 26, 32, 36, 40]. In order to elucidate the mechanisms of electric-field stimulation on osteoprogenitor cells, many *in vitro* studies have applied direct electrical currents [8, 12, 29, 37, 41], capacitive coupling of electric fields [16, 28, 42] and inductive coupling of electric fields [9, 19]. Ochi *et al.* showed that the bone contact ratio around a dental implant inserted in the rabbit femur was almost 70% when capacitive coupling of electric fields (60 kHz, 10 Vp-p, sine wave signals) was applied for 8 hr/day over 14 days [32]. Sato *et al.* showed that PLLA braided artificial tendons implanted in rabbit Achilles tendons had good initial strength and showed increased ingrowth of newly formed fibrous tissue [34]. Although the underlying mechanisms have not yet been completely elucidated at a molecular level, the results of these studies suggest that electric stimulation accelerates cell proliferation, differentiation into osseous tissue and matrix synthesis. In this study, patellar ligament was selected to ap-

Fig. 4. Histological evaluation with hematoxylin and eosin staining. A: At 2 weeks after surgery for the helical PLLA group. The right upward blank cavity shows the cross section of the PLLA fiber. The surface of the regenerated tissues was smoothly connected to the PLLA fiber (arrows). Newly formed osseous tissues were observed (*). B: At 2 weeks after surgery for the control group. The surface of the regenerated tissue was rough (arrows). C: At 4 weeks after surgery for the helical PLLA group. Arrow shows interface between regenerated osseous tissue and PLLA fiber. D: At 4 weeks after surgery for the control group. Bars represent 100 μ m.

Fig. 5. Comparison of the mean contact ratio of the osseous tissue to the PLLA fibers. The contact ratio of the osseous tissue around the PLLA fibers is given by the circular arc length of the osseous tissue (a) divided by the circumferential length of the PLLA fiber (C). (*: $P < 0.05$, **: $P < 0.01$).

ply repeated load on the PLLA fibers *in vivo*. Although there are no data in rats, the peak tensile stress of patellar ligament during gait is reported 230% of body weight in rabbits [38]

and 200% of body weight in goats [24]. It may be suggested that the peak tensile stress applied in the PLLA fiber-grafted patellar ligament in the current study was almost 4.9 N (500

gf). The loaded fiber passed through the hole drilled in the patella and tibia (Fig. 2) where enhanced bone contact ratio around the helical PLLA fibers at 4 weeks after surgery was observed. Although there are some possible explanations for osteogenic stimulation, such as micromovement, viscoelasticity or tissue reaction to the PLLA fibers, it is likely that the piezoelectric potential generated by the helical PLLA fibers was a dominant factor for the increased bone formation, because the micromovement, viscoelasticity and tissue reaction of helical PLLA fibers were almost the same as those of non helical fibers. The results of the current study suggest that the helical PLLA fiber may be useful for the bioabsorbable surgical suture or artificial ligament to better connect with the bone. In the current study, we did not examine the degradation rate of implanted PLLA fiber. Laitinen *et al.* reported that PLLA fiber, 2.0 mm in diameter, retained 74 and 22% of initial tensile load carrying capacity at 6 and 12 weeks after the subcutaneous implantation in rabbits, respectively [25]. Bernstein *et al.* demonstrated that cell adhesion rate of PLLA/ β -tricalciumphosphate composite material is higher than PLLA alone [4]. In the strategy of regenerative therapy using biodegradable ligament, cell adhesiveness and degradation speed are important parameters for histological tissue regeneration and biomechanical tissue regeneration. In the future, developmental research about these parameters should be performed to create a clinically-effective helical PLLA artificial ligament.

In conclusion, PLLA fibers with helical structure generate a piezoelectric charge under cyclically applied tensile stress at a frequency of 7 Hz. Significantly higher ossification was observed around the implanted helical PLLA fibers in the rat knee joint, compared to control fibers.

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