J Mater Sci: Mater Med (2009) 20:1175–1180
DOI 10.1007/s10856-008-3646-4

Evaluation of a multi-layer microbraided polylactic acid fiber-reinforced conduit for peripheral nerve regeneration

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Received: 1 April 2008 / Accepted: 3 November 2008 / Published online: 30 December 2008

Abstract We evaluated peripheral nerve regeneration using a biodegradable multi-layer microbraided polylactic acid (PLA) fiber-reinforced conduit. Biodegradability of the PLA conduit and its effectiveness as a guidance channel were examined as it was used to repair a 10 mm gap in the rat sciatic nerve. As a result, tube fragmentation was not obvious and successful regeneration through the gap occurred in all the conduits at 8 weeks after operation. These results indicate the superiority of the PLA materials and suggest that the multi-layer microbraided PLA fiber-reinforced conduits provide a promising tool for neuro-regeneration.

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1 Introduction

To repair an injury-induced nerve defect, both ends of the injured nerve stumps can be introduced into a tubular chamber, which can offer the advantages of minimizing invasion and scarring of the nerve, aiding guidance of growing fibers along appropriate paths by mechanical orientation and confinement, and enhancing the precision of stump approximation. Several synthetic materials, either nondegradable [1] or biodegradable [2–4], have been used as a nerve conduit. In these materials, polylactic acid (PLA), which is made from corn, sugar beets or wheat, is an important material in the medical industry that has been used for over 30 years. It is a polymer that has good biocompatibility, which can satisfy the request for tissue regeneration and repair [5–7]. It is also a biodegradable polymer that can be assimilated by the body, and therefore has been largely used in sustained-release drug delivery systems [8, 9].

Several years ago, my group first fabricated a rotor-twister which could twist multifilament to increase yarn strength [10, 11]. Using this machine, enormous advances have been made in recent years in the development of PLA suture materials. We found that the PLA surgical suture composite with chitosan could inhibit bacterial growth and promote wound healing [12, 13]. However, these PLA suture materials have a low mechanical strength under physiological conditions, thus limiting their applications. To obtain adequate mechanical strength, we describe a method of preparation of PLA nerve conduits that uses twisted PLA filaments to fabricate a multi-layer tubular construct by microbraiding technique for reinforcing a nerve bridge across a 10 mm gap for rat sciatic nerves.

Temporal and spatial progresses of tissue activity within the conduit were studied. Its biocompatibility and effectiveness as a guidance channel for peripheral nerve regeneration were evaluated.

2 Materials and methods

2.1 Fabrication of microbraided PLA nerve guide conduits

Four PLA filaments (Unitika, Japan) were inserted into the rotor-spinning device driven by the tangent belt of the motor shown in Fig. 1. The ends of the filament were pulled by the take-up roller. The filament was then twisted by rotation of the rotor-twister. Sixteen twisted PLA filaments were wound on the spindles of the microbraiding machine shown in Fig. 2. The ends of the filament from the spindles were then pulled to the center of the machine. A silicone rubber mandrel of outer diameter 1.74 mm was inserted through the convergence point and forming point of the microbraiding machine from the bottom and pulled upward. The machine was then switched on to braid, and a singe-layer tubular structure of infinite length was obtained. After repeating the foregoing method, a double- and a triple-layer braiding tube were then constructed. The braiding tube was then cut to 12 mm and both of its ends were melted with a heated penknife to prevent the filaments from unwinding and the mandrel removed. The tubes were then immersed into 0.02 M NaOH, cleaned by an ultrasound oscillator for 1 h, rinsed with distilled water, and dried in an oven at 60°C for 30 min. Finally, the tubes were sterilized with 25 kGy of γ-ray for subsequent implantation.

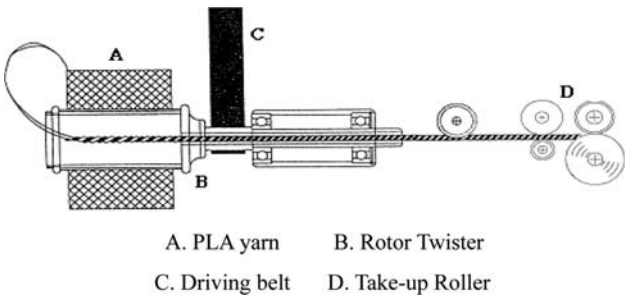

Fig. 1 The mechanism of rotor-twister

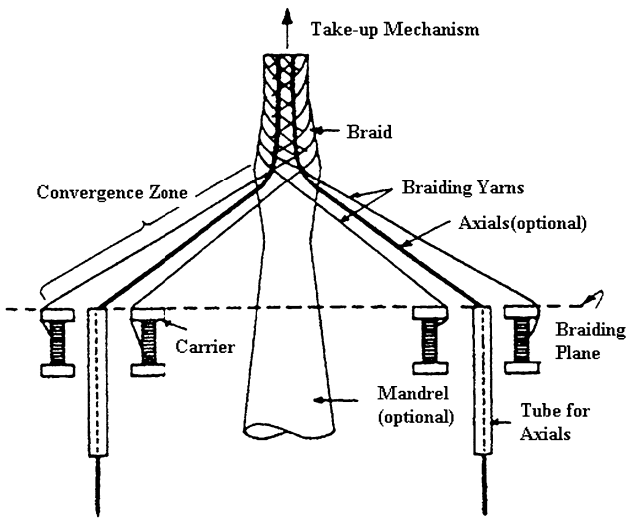

Fig. 2 Braiding machine apparatus

2.2 Microscopic observation of microbraided PLA nerve guide conduits

To examine the morphology of the microbraided PLA nerve guide conduits with scanning electron microscopy (SEM), the samples were gold-coated using a Hitachi E-1010 Ion Sputter and micrographs were obtained using a Hitachi S3000 N scanning electron microscope (Hitachi High-Technologies Co., Tokyo, Japan) at an accelerating voltage of 5 kV.

2.3 In vivo evaluation of the subcutaneous implantation in rats

In this experiment, we used nine healthy adult male Sprague–Dawley rats, weighing approximately 200–300 g. For the insertion of the implants, incisions (0.5 cm in length) were made and single-, double-, and triple-layers of PLA nerve guide conduits were randomly implanted subcutaneously on both sides of the rats. Each rat received six implants, which were removed upon sacrifice at various times: 1, 2, and 4 weeks. At each implantation time, three rats were operated on. The implants were removed and the tissue-covered implants were prepared for histological evaluation. All animals were maintained in facilities approved by the China Medical University for Accreditation of Laboratory Animal Care, according to the regulations and standards of the National Science Council of Health of the Republic of China.

2.4 Histological evaluation

After retrieval, the tissue-covered implants were fixed in 10% formalin (Merck, Whitehouse Station, NJ) for 2 days. Tissue was rinsed in normal saline (Taiyu, Hsin-Chu, Taiwan) and dehydrated in a series of graded alcohols (50, 70, and 95%; Merck, Whitehouse Station, NJ) for 30 min each. Samples were then embedded in paraffin (Merck, Whitehouse Station, NJ) and cut into thin 12 μm sections by using a microtome with a dry glass knife. For histomorphometric evaluation, sections were stained with

hematoxylin and eosin (Sigma Chemical Co., St. Louis, MO). The tissue reactions to the implants in the subcutaneous tissue were evaluated on the basis of the uniformity and thickness of the foreign body capsule as well as the inflammation responses under an optical microscope (Olympus IX70, Olympus Optical Co., Ltd., Japan).

2.5 Microbraided PLA nerve guide conduits implantation

Thirty adult Sprague–Dawley rats underwent placement of single-, double-, and triple-layers of PLA nerve guide conduits, which were removed upon sacrifice at the time point of 8 weeks. For each type of nerve guide conduits, 10 rats were operated on. The animals were anesthetized with an inhalational anesthetic technique (AErrane®, Baxter, USA). Following the skin incision, fascia and muscle groups were separated using blunt dissection, and the right sciatic nerve was severed into proximal and distal segments. The proximal stump was then secured with a single 9-0 nylon suture through the epineurium and the outer wall of the PLA nerve guide conduits (1.74 mm ID). The distal stump was secured similarly into the other end of the chamber. Both the proximal and distal stumps were secured to a depth of 2 mm into the chamber, leaving a 10 mm gap between the stumps. The muscle layer was re-approximated with 4-0 chromic gut sutures, and the skin was closed with 2-0 silk sutures. All animals were housed in temperature (22°C) and humidity (45%) controlled rooms with 12 h light cycles, and they had access to food and water ad libitum.

2.6 Electrophysiological techniques

All the animals with apparent nerve regeneration were re-anaesthetized and the sciatic nerve exposed. The sciatic nerve was stimulated with supramaximal stimulus intensity through a pair of needle electrodes placed directly on the sciatic nerve trunk, 5 mm proximal to the transection site. Amplitude, latency and area of the evoked muscle action potentials (MAP) were recorded from gastrocnemius muscles with micro-needle electrodes linked to a computer system (Biopac Systems, Inc., USA). The latency was measured from stimulus to the takeoff of the first negative deflection. The amplitude and the area under the MAP curve from the baseline to the maximal negative peak were calculated. The MAP was used to calculate the nerve conductive velocity (NCV), which was carried out by placing the recording electrodes in the gastrocnemius muscles and stimulating the sciatic nerve proximally and distally to the PLA nerve guide conduits. The NCV was then calculated by dividing the distance between the stimulating sites by the difference in latency time.

2.7 Histological techniques

Immediately after the recording of muscle action potential, sciatic nerve sections were taken from the middle regions of the regenerated nerve in the chamber. After the fixation of glutaraldehyde (Merck, Whitehouse Station, NJ) the nerve tissue was post-fixed in 0.5% osmium tetroxide (Sigma Chemical Co., St. Louis, MO), dehydrated in a series of graded alcohols (70, 80, 95, and 100%; Merck, Whitehouse Station, NJ) for 60 min each, and embedded using a JB-4® embedding kit (Polysciences Inc., Warrington, PA). The tissue was then cut to 5-μm-thickness by using a microtome with a dry glass knife, stained with toluidine blue (Sigma Chemical Co., St. Louis, MO). Using an optical microscope (Olympus IX70, Olympus Optical Co., Ltd., Japan) with an image analyzer system (Image-Pro Lite, Media Cybernetics, USA), the neural components in each nerve section was observed.

2.8 Statistical analysis

All the measurements were done by the same observer and data expressed as mean ± standard deviation. Statistical comparisons between groups were made by the one-way analysis of variance (SAS 8.02). The Tukey test was then used as post hoc test.

3 Results

3.1 Microscopic observation of microbraided PLA nerve guide conduits

Figure 3a–c show the micrographs of the microbraided PLA nerve guide conduits with different layers. Porous surface with crosslinking fibers were seen. The pore size of the pores found in between the crosslinking of the fibers was decreased as the layer of the conduit was increased.

3.2 Biocompatibility of microbraided PLA nerve guide conduits

No clinical problems were seen for any of the rats in the postoperative period. At 1 week post-implantation, all the PLA nerve guide conduits with different layers persisted maintaining their lumens and wall integrity. An acute inflammatory response was characterized by a rapid accumulation of cells resembling lymphocytes and macrophages at the site between conduits and their surrounding tissue (Fig. 4a). At 2 week, a delicate and thin

Fig. 3 SEM micrographs of the single- (**a**), double- (**b**), and triple-layer (**c**) microbraided PLA nerve guide conduits

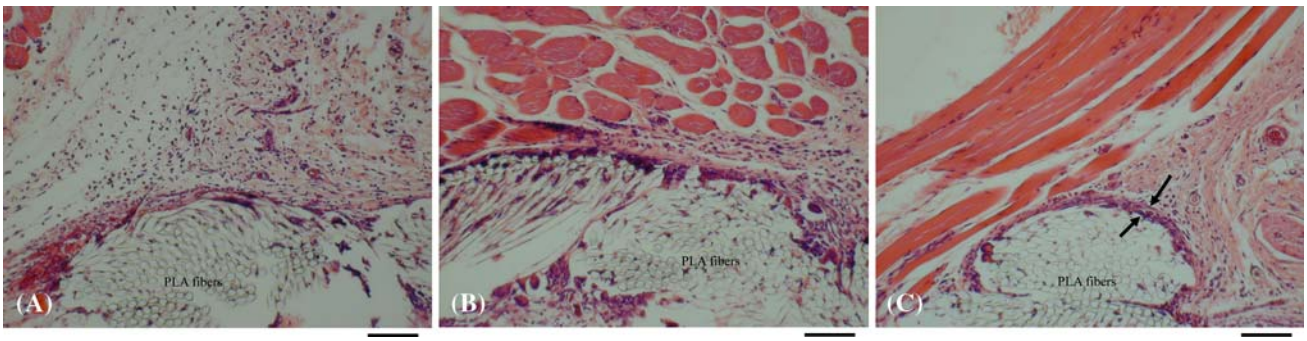

Fig. 4 Tissue sections, after PLA nerve guide conduits were implanted for 1 week (**a**), 2 weeks (**b**), and 4 weeks (**c**). Arrows show the site of foreign body capsule. Scale bars = 100 μm

fibrous tissue capsule (<20 μm in thickness) was present surrounding the whole implant (Fig. 4b). Inflammation responses were still obvious with abundant inflammatory cells. Neocapillaries were seen dispersing within the fibrous tissue capsule. At the time point of 4 weeks (Fig. 4c), fibrous tissue capsules became thicker with a compact structure along with active neovascularization. Up to this time, the inflammatory reaction decreased remarkably. A chronic inflammation reaction was noted with macrophages and giant cells getting around the edges of the PLA nerve guide conduits.

3.3 Electrophysiological measurements

MAPs were recorded at 8 weeks of post-operation. Measurements of latency, peak amplitude, area under the MAP curves, and the NCV were calculated for each nerve (Fig. 5a–d). As a result, the difference of all the

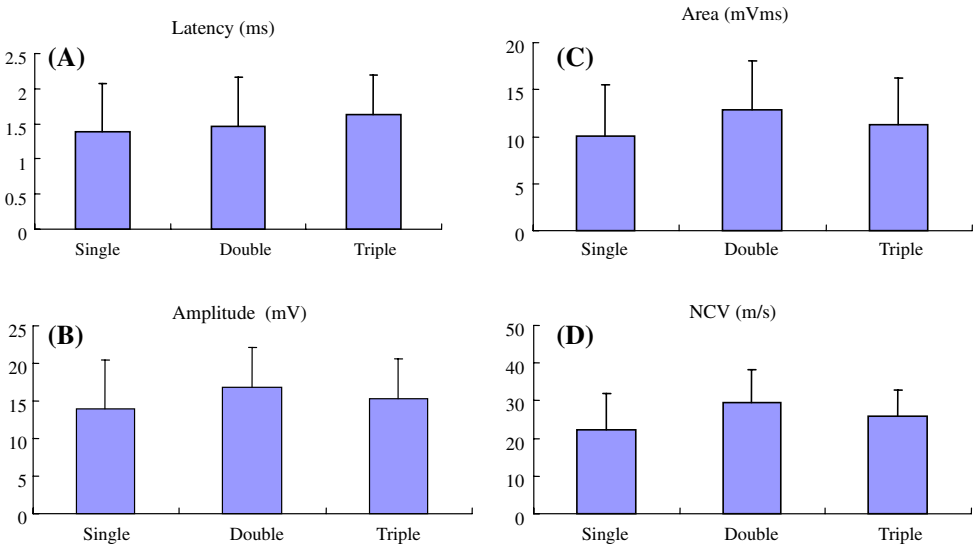

Fig. 5 Analysis of the evoked muscle action potentials, including latency (**a**), amplitude (**b**), area under the MAP curves (**c**), and NCV (**d**)

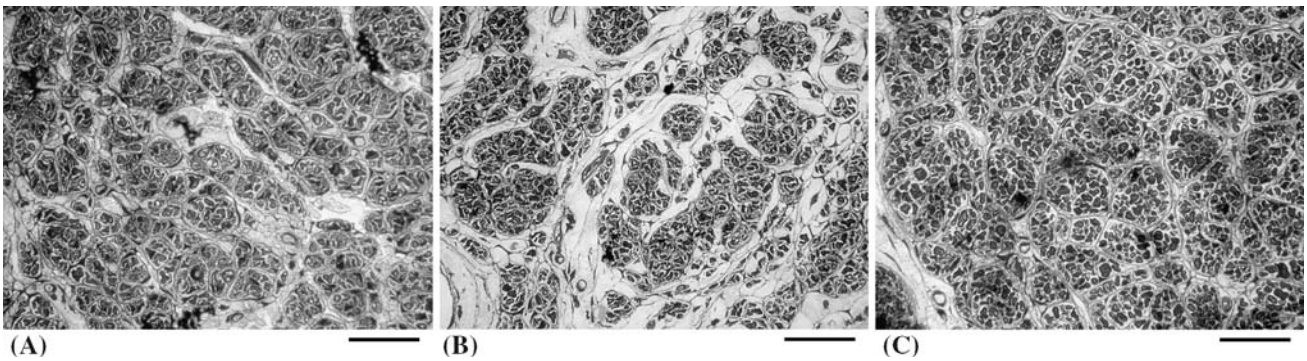

Fig. 6 Light micrographs of regenerated nerves retrieved from the single- (**a**), double- (**b**), and triple-layer (**c**) microbraided PLA nerve guide conduits. Scale bars = 30 μm

measurements among the nerves obtained from the PLA nerve guide conduits with different layers did not reach the significant level at $P < 0.05$.

3.4 Nerve regeneration

Throughout the 8 weeks of experimental period, swelling or deformation of all the PLA nerve guide conduits was not seen. Brownish fibrous tissue encapsulation was noted, covering all over the PLA nerve guide conduits and the parts of the nerve stumps in the tube openings. After trimming the fibrous tissue, cutting the wall of the tube, the regenerated nerve was exposed and then retrieved. Overall gross examination of the single-, double-, and triple-layers of PLA nerve guide conduits all revealed 100% of nerve formation in the tubes.

In the micrographs, the regenerated nerves retrieved from the PLA nerve guide conduits with different layers displayed a similar structure with a thick fibrous tissue, surrounding a cellular and vascularized endoneurium (Fig. 6a–c). Schwann cells organized in clusters surrounding groups of unmyelinated axons were also present. These axon-Schwann cluster formations, termed as regeneration units, are common organization structures seen under nerve cuff bridging conditions [14–16]. In addition, Schwann cell columns were also seen, which may participate in the early scaffold formation for the migration of advancing axonal tips.

4 Discussion

The main objection for using non-degradable conduits is that they remain in situ as foreign bodies after the nerve has regenerated. To avoid a second surgery to remove the implant and the potential confounding factor of post-surgical infection, biodegradable materials seem a more promising apparatus to reconstruct nerve gaps. However, swelling of degradable tube walls caused by absorption of body fluids can happen during the nerve regenerative processes [17]. The swelling could occlude the lumen and therefore impair axonal regeneration. Therefore, materials for the fabrication of nerve guides are required to be degrading properly without obvious swelling, as well as suitable mechanical properties to provide longitudinal support for the regenerating nerves [18].

In order to obtain adequate mechanical strength, a number of techniques have been used to cross-link the nerve guide materials, i.e., thermal heating, ultraviolet irradiation, and mostly chemical cross-linking by cross-linking agents such as formaldehyde and glutaraldehyde [19]. However, these synthetic cross-linking agents are highly cytotoxic [20]. Therefore, we used twisted PLA filaments to fabricate a multi-layer tubular construct by microbraiding technique to reinforce the nerve bridge. As a result, we found that successful regeneration of nerves across the gap occurred in all of the PLA nerve guide conduits with different layers. Macroscopic observations showed that unsatisfactory swelling or deformation of the PLA nerve guide conduits was not seen. We believe that the stable dimensions of the PLA nerve guide conduits played a critical role in contribution to the high success of nerve regeneration. The porous structure of the microbraided conduit had the required permeability to allow for the passage of nutrients for nerve regeneration from the external environment into the conduit lumen. The thin layer of surrounding fibrous tissue and minimal inflammation reaction also indicated that the PLA nerve guide conduits were biocompatible. These results are not surprising since the polylactic acid has been shown a promising material for use in entubulization repair of nerve defects [21, 22].

Though these aforementioned results are encouraging; however, the size of the pores found in between the crosslinking of the PLA fibers was decreased as increasing the layer of the conduit. We were afraid that the decreasing pore size of the conduit could not provide enough space to allow the exchange of fluids and nutrients in the tubal

lumen. Fortunately, we did not see deleterious effects on nerve function caused by the change of pore size in the present study. No differences were seen for the electrophysiological measurements and histological observations of the nerves obtained from the PLA conduits with different layers.

5 Conclusion

The present study shows that the multi-layer PLA nerve conduits manufactured by microbraiding technique are successful in having cables bridge a 10 mm gap in the sciatic nerve of the rat. The temporal and spatial progresses of cellular activity within the conduit are similar to those seen for experiments using biodegradable nerve guides reported in the literature [23–25]. Since the properties of the multi-layer PLA nerve conduits are stable that further study on longer gap length is applicable.

Acknowledgements The authors would like to thank National Science Council of the Republic of China (Contract No. 96-2628-E-039-011-MY3, 96-2622-E-039-001-CC3) for financially supporting this research. Dr. Tsai-Chung Lee is appreciated for her assistance in analyzing the data.

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