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A SHAPE MEMORY NERVE CONDUIT FOR PERIPHERAL NERVE REGENERATION

Cheng Chen[1], Jinlian Hu[*2], TingwuQin[3], WutianWu[4], JieCao[5] & Yunhu Tue[6]

[1&*2]Institute of Textile & Clothing, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

[3]Institute of Stem Cell and Tissue Engineering, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, China

[4]Department of Anatomy, Li KaShing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China

[5]Southern Medical University, Guangzhou, China

[6]Zunyi Medical College, Zunyi 563003, China

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ABSTRACT

Nerve repair and regeneration have been a unique clinical challenge for surgeons. In this study, we proposed a smart nerve conduit that can simplify the surgery process and achieve peripheral nerve regeneration by automatic gradual lengthening.To determine the in vivo role of shape-memory nerve conduit for peripheral nerve regeneration, the fabricated conduits were implanted in animal models for 1, 2, and 3 months, respectively. Based on the experimental data, the shape-memory conduit could be used as potential scaffold for peripheral nerve regeneration. Further improvements for satisfactory performance are suggested.

I. INTRODUCTION

Thousands of peripheral nerve injuries occur throughout the world as a result of traffic accidents, natural disasters and other types of trauma every year. Selecting the proper treatment has been a challenging clinical problem for surgeons.[1,2]Besides direct end-to-end repair and nerve grafts, the development of nerve conduits to enhance nerve regeneration remains a promising research area for improving patient outcomes.[3,4]Existing conduits for nerve regeneration have focused on aiding guidance using biocompatible polymeric scaffold such as those based on polyglycolic acid and poly(DL-lactide-ε-caprolactone) or based on natural polymers such as collagen.[5,6] Shape memory polymers (SMPs), which are a class of stimuli-responsive materials being able to revert to the pre-defined shape upon application of external stimulus, have been applied in biomedical field.[7] A variety of SMP-based biomedical devices have been designed and developed, such as self-tightening sutures, and laser actuated SMP microgripper.[8,9]

Herein, we introduced a smart fibrous nerve conduit that may achieve desirable peripheral nerve regeneration by prolonged automatic gradual lengthening, as shown in Figure 1.To achieve gradual shape-memory effects, Huang demonstrated gradual shape recovery function and found that the recovery time is determined by the temperature gap between T_g and the ambient water temperature, the recovery process of a polymer with lower T_g starts and ends earlier. Instead of synthesizing a SMP with gradient T_gs, we hereby design a feasible approach for gradual-recovery purpose, by ejecting a series of polymer solutions with different T_gs in sequence. We can manufacture a conduit with gradient T_gs in macroscopic level.SMP often consists of two segments: the switching one for the fixation of temporary shape and the hard one for the fixation of the permanent shape. As for hard segment, covalently cross-linking presents certain advantages in mechanical behavior while simultaneously limiting many aspects of materials processing capability. In this study, to meet the specific requirements of electrospinning fabrication and biodegradability, we employ the entanglements of the molecular chain to function as physical cross-links.A series of high molecular weight poly(*rac*-lactide-*co*-glycolide) with different T_gs were synthesized through ring-opening polymerization.

The shape-memory functionality of the synthesized copolymers was quantified in cyclic thermo-mechanical experiments between 20℃ and 60℃.Figure 2 present the thermo-mechanical experiments of PLGA-60/40, PLGA-40/60, and PLGA-20/80. For all networks, the strain fixity ratio R_fs were near 99% under stress-free conditions, even after five thermo-mechanical cycles. These good shape-fixities could prevent uncontrolled implant deployment during implantation.The gradual increase in recovery ratio R_r with increasing testing cycle indicates that these copolymers can recovery from deformation and return to their original shape and our proposed physically cross-linked SMP possesses a loose net-points structure.To demonstrate the SMP system's feasibility for fabricating a smart nerve conduit, a prototype of nerve conduit device is designed, manufactured, and characterized in this work. A prototype of tri-segment conduit, which was made from three types of high molecular weight amorphous poly(*rac*-lactide-*co*-glycolide) copolymers, was fabricated by electrospinning technology, as shown in Figure 1B.

The surface morphology of each segment was observed via SEM, as shown in Figure 2. The diameter of electrospun fibers is of similar magnitude as that of fibrils in extracellular matrix (ECM) that mimics the natural tissue environment and has presented effectiveness as a substrate for cell growth.In addition, the physical form of the electrospun nerve conduit such as porosity can greatly influences its biocompatibility with tissue *in vivo*.[10]Usually, biologic nerve grafts made from autograft/allograft are revascularized within the first 4-5 days after implantation by longitudinal ingrowth of vessels from the distal and proximal nerve stump and sprouting of collateral capillaries.[11] This process requires diffusion of nutrients, growth factors and other biologically active agents into the area of nerve regeneration. Therefore, porous conduit can allow the influx of external nerve regeneration factors and the outward diffusion of waste products.[12,13]In addition, porous nerve tube may also facilitate the formation of a supportive fibrin by allowing inward diffusion of local or systemic healing factors.
To demonstrate the ability of PLGA copolymers to support the adhesion and proliferation of Schwann cells and the production of extracellular matrix, cell culture studies were performed. Cell morphology on the different types of material surfaces was utilized to determine the cytocompatibility of the synthesized copolymers. In Figure 3, the attachment of Schwann cells on the surfaces of PLGA-60, PLGA-40, and PLGA-20 electrospun films is quantified after culturing for 6h, 24h, and 48h culturing based on the confocal microscopy images showing DAPI staining. In each case the number of viable Schwann cells increased in time, indicating that all the surfaces allow the growth and proliferation of Schwann cells.Figure 3 shows the morphology of Schwann cells cultured on the three types of PLGA nanofibrous films, and revealed that Schwann cells flattened and extended in a sequential fashion as cultured for 6, 24, and 48 h. The whole process of adhesion and spreading consists of cell attachment, filopodial growth, cytoplasmic webbing, and flattening of the cell mass that are performed in a sequence.

In this study, adult New Zealand white rabbits approximately 2 months of age were used to evaluate the *in vivo* biocompatibility and the nerve regeneration performance. Briefly, the animals were divided into 3 groups each with 6 rabbits. Group A: elongated nerve conduits; group B: nerve conduits with original length; group C: autograft nerve group as a positive control.Then, the sciatic nerves of animal models were resected to obtain a 15 mm nerve gap under a standard surgical procedure. Subsequently, both the proximal and the distal stumps were sutured with the elongated/original conduits, leaving a 15 mm gap between the stumps. In a similar microsurfical technique, nerve autografts were used to repair defects in the rabbit sciatic nerve. Nerve autografts were placed in a reverse fashion to prevent axonal branching during proliferation through side branches from the donor nerve. Therefore, each rabbit received one implant and histomorphology evaluation was performed to evaluate the *in vivo* performance, as shown in Figure 4.Hematoxylin and eosin (HE) stain is one of the principal stains in histology. Hematoxylin binds to basophilic substances (such DNA/RNA in the nucleus, which are acidic and negatively charged) and stains them violet. Eosin binds to acidophilic substances (such proteins in the cytoplasm, which are basic and positively charged) and stains them pink.[14-16] Moreover, the stained proteins could include cytoplasmic filaments in muscle cells, intracellular membranes, and extracellular fibers. Masson's trichrome is suited for distinguishing cells from surrounding connective tissue, which stains keratin and muscle fibers red, collagen and bone blue, cytoplasm pink, and cell nuclei black. It is widely employed to study muscular dystrophy, cardiac infarct or kidney pathologies.

To study the *in vivo* biocompatibility, we evaluated the toxicity of nerve conduit based on the implantation time of poly(*rac*-lactide-*co*-glycolide)-based conduit. Since the degradation of polymeric biomaterials may affect the tissue in several ways,the implanted polymeric biomaterials will gradually release various chemical products of degradation, including common additives, impurities, monomers, and oligomers. These chemical components would introduce types of toxic reactions in the tissues by slowly migrating from the interior to the surface and

the surrounding tissue, causing inflammation or pathologic changes in the tissues of interest.[17] Briefly, 12 weeks post-implantation, excised important organs from heart, liver, spleen, and kidney were frozen and embedded in paraffin wax, were sectioned into 8 um slices, were stained by hematoxylin/eosin (HE) stain method, and were observed by microscopy to fully evaluate the impact of the chronic exposure of conduit in the animal model. As shown in Figure 5, the implantation of the fabricated nerve conduit did not cause any pathologic damage to rabbit heart, liver, spleen, or kidney, indicating good *in vivo* biocompatibility.

To study the efficiency of nerve conduits for nerve regeneration, at week 4, 8, and 12, the regenerated nerves were cut into 5 um thick longitudinal sections that were then stained with Masson's staining, respectively to detect structures. As shown in Figure 6, the appearance of the polymeric implants and surrounding tissue varied throughout the course of implantation.Masson staining images of the nerve longitudinal sections from experimental groups reveal the absence of an acute immune response in close proximity to the fibers, indicating the noninflammatory nature of the fabricated PLGA fibers. These results noticeably support efficacy of the fabricated tri-segment conduit to be biocompatible *in vivo* model.At week 8 and 12, obvious fibrosis and scarring was observed in the tissue surround elongated conduit. In addition, during the nerve regeneration process, the structure of nerve conduit was stably maintained. This is an important factor for axon growth.Although the results of *in vivo* biocompatibility and shape-memory effect are encouraging, the nerve regeneration results are less than those noted with control autografts.We believe the fabricated nerve conduit can serve as a viable scaffold for axonal guidance, but modifications of the biological environment with support cells and nerve induction factors may enhance nerve regeneration performance.

II. CONCLUSION

In summary, the purpose of this study was to propose a potential smart nerve conduit based on shape-memory polymer for peripheral nerve regeneration. The results noticeably support the efficacy of the fabricated tri-segment conduit to be biocompatible *in vivo* model. Specifically, compared to the normal nerve conduit, the shape-memory conduit could introduce the automatic recovery function and facilitate peripheral nerve regeneration physically to some extent. Nevertheless, although the fabricated conduit offers several advantages including its biocompatibility, porous structure and consistency in design requirements, the nerve regeneration efficiency still needs further improvement because the equivalent nerve regeneration to autograftshas not yet achieved. We will further explore the optimized recovery in our next study. For example, to further mimic the natural regenerative microenvironment, various support cells and/or growth factors could be introduced into our fabricated SNC to improve the regeneration effect. Schwann cells are the main myelin-forming cells in the peripheral nervous system and play a prominent role in neuron regeneration. Meanwhile, nerve growth factor (NGF) is a neuropeptide primarily involved in the maintenance, proliferation, and survival of certain target neurons. Therefore, the aforementioned cells and growth factors could be potential candidates to be filled in our designed SNC.

III. ACKNOWLEDGEMENTS

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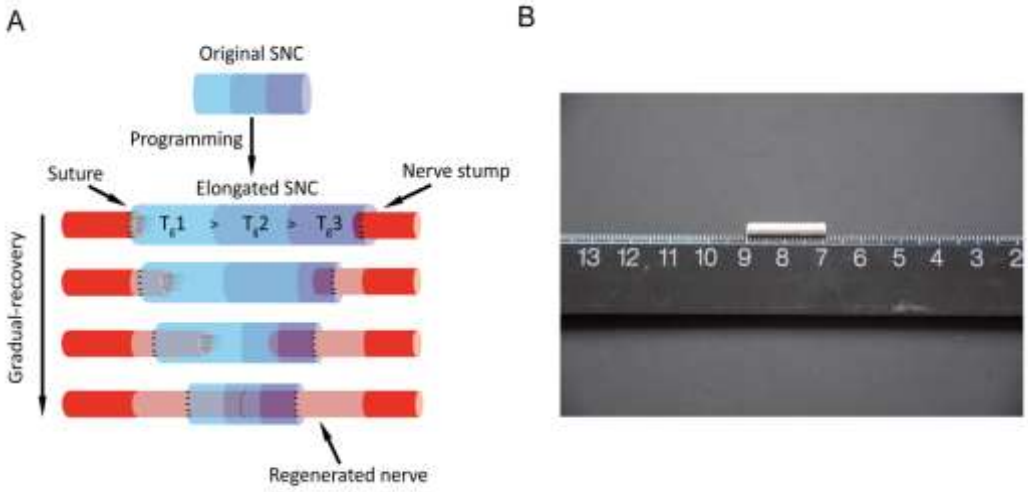

Figure 1. (A) Working concept of a water-triggered shape-memory nerve conduit. A tri-segment conduit with gradual-recovery function can be fabricated by electrospinning. After being implanted *in vivo* and triggered by water, the elongated conduit can recovery to the original shape.(B) Permanent shape of a tri-segment smart nerve conduit.

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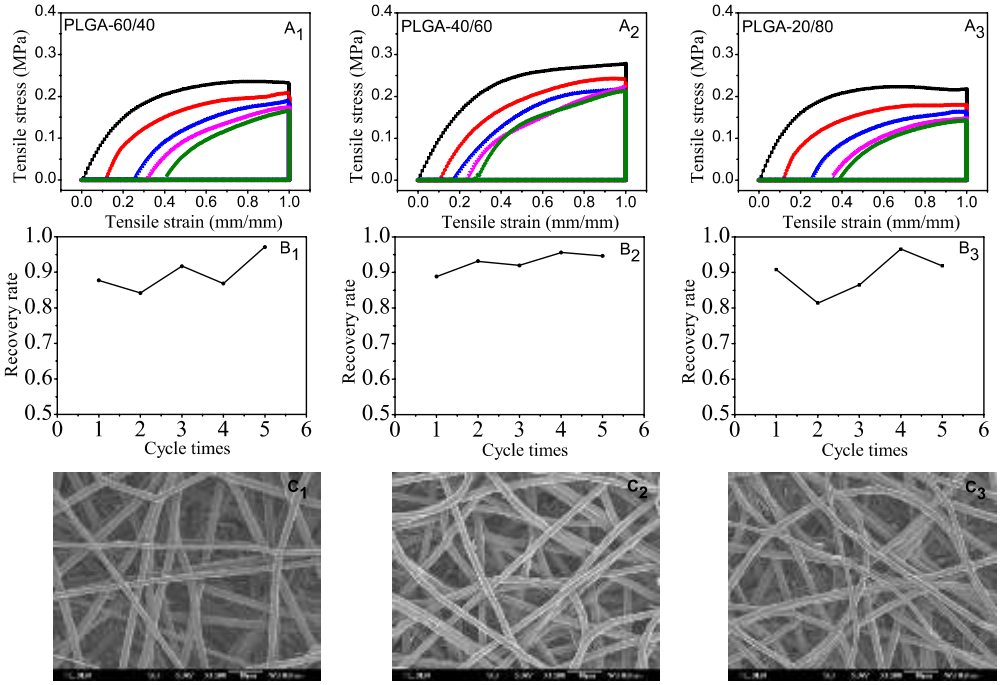

Figure 2. (A_1-B_3) Materials characterization data demonstrating the shape-memory effects, (C_1-C_3) SEM micrographs of electrospun nerve conduit fibers.

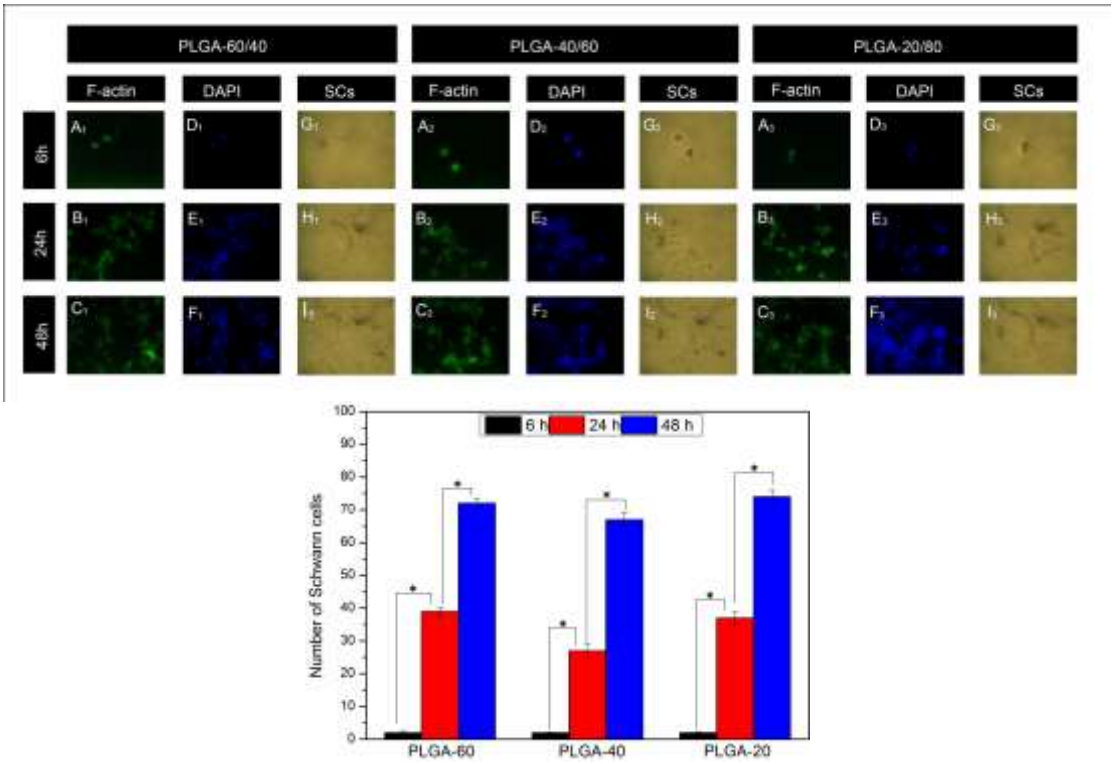

Figure 3Fluorescent microscope images and optical microscopic examination of Schwann cells cultured on three types of fabricated electrospun scaffolds for 6, 24, and 48 h. Image sets A-C and D-F represent F-actin (green) and nucleus (blue) were stained by FITC-Phalloidin and DAPI, respectively. Image sets G-I represent the morphology of Schwann cells. The number of Schwann cells attached to the surface of PLGA-60, PLGA-40, and PLGA-20 electrospun films after 6 h, 24 h, and 48 h culturing

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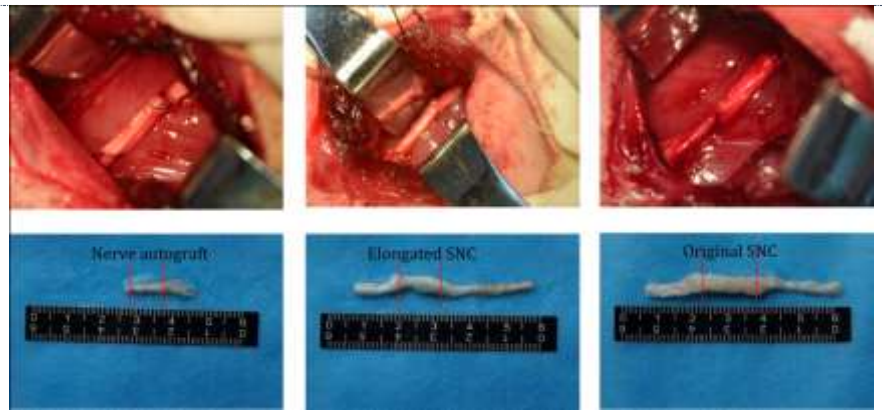

Figure 4The gross view of sciatic nerve regeneration with different nerve grafts.

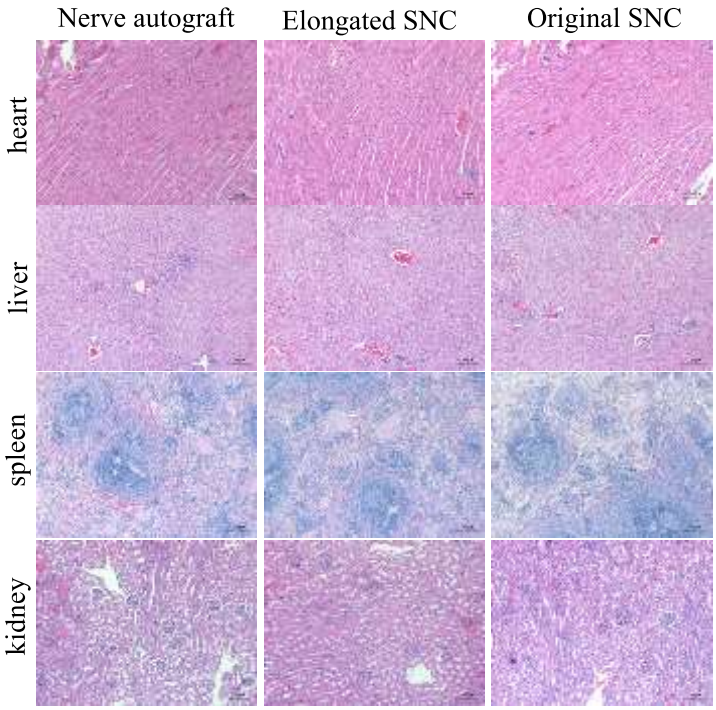

Figure 5. HE staining of important organs of heart, liver, spleen, and kidney

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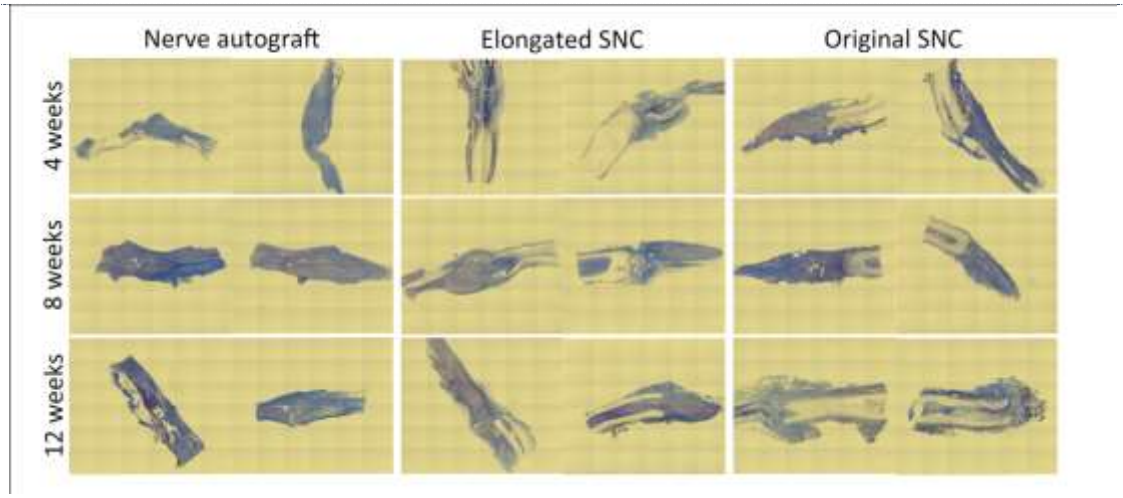

Figure 6 Longitudinal section of regenerated nerves taken from types of nerve conduits implanted in rabbit for 4 weeks, 8 weeks, and 12 weeks stained with Masson

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