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Sciatic Nerve Regeneration in Mice Using A PLGA Microgroove Patterned Conduit Fills with Microfiber

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Abstract – Nerve conduit is one of common strategy for peripheral nerve repair in clinical, but the repairing process is slow and complex leading to a poor result of functional recovery. From neuroanatomy inspiration, a nerve conduit combine with micro groove patterned and microfiber for guiding nerve regeneration in animal test was evaluation in this study. The master mold of poly(lactic-co-glycolic acid) microgroove patterned membrane and microfiber were manufactured by photolithography and poly(dimethylsiloxane) casting. A bunch of microfiber was wrapped with micro-patterned membrane forming a filler type conduit (Conduit 2.0). The microgroove pattern only conduit was used as control group (Conduit 2.1). Sample was then implanted into injured FVB mice sciatic nerve for 8 week to emulate the trauma recovery. According to the SEM image, these micro structures were not degradation after 8 weeks implantation. The neurite outgrowth and cell migration on conduit can be modulated by mechanical causes of surrounding environment. However, some fragmentation microfibers were observed to disrupt the cell migrating direction, and interfered the guiding ability of microgroove patterned on inner wall. In Rotarod test, the mouse implanted using the conduit with microfiber showed a worse result compare to micro pattern only conduit at early stage, but did not show difference at final stage. The filling type conduit presented as dramatically poor recovery on the compound muscle action potential measurement after 8 weeks implantation, since the microfiber occupied most space leading to a lower mass transportation rate. In conclusion, microfiber filled conduit is a good tool for guide cell migration and neurite extension, but the degraded fragment and filling density still need to settle up to increase mass transportation inside conduit.

Keywords**:** Microgroove Pattern, Microfiber, Nerve Conduit, Peripheral Nerve Regeneration.

1. Introduction

Damage to the nerve system, caused by mechanical, thermal, chemical, or ischemic, can disrupt the axonal connection between neural cell bodies and innervated tissue and impair various functions. The peripheral nerve system can spontaneously regenerative, when suffer a minor damage. However, if the damage is too extensive with an acute extracellular matrix (ECM) disruption, a bridge is introduced to reconnect the nerve stump. In clinical, the autograft insertion has been known as golden standard for repairing peripheral nerve injury. Autograft provides a good microenvironment conductive to regenerative, but an additional surgery is needed to harvest a nerve segment from donor site which leading to tissue morbidity at this site. Hence, alternative synthetic nerve conduits are developed to recover the injuring nerve into an optimal level.

The hollow nerve conduit which clinically uses to bridge the nerve defect by fixing the proximal and distal end of nerve stump into the respective end of the tube is approved. However, the hollow nerve conduit only has been shown a successful clinical results to improve nerve reconnection for a subclinical gap injury (3-10 mm) [1]. Therefore, the synthetic nerve conduit which mimicking the native microenvironment of nerve to provide the surpassing properties than using autologous nerve graft for repairing are widely discussed. An ideal nerve conduit should provide good ability to direct axon growth orientation when neurite extension. The fibrous intraluminal fillers and the structurally patterned intraluminal are commonly designs on conduit to guide neurite extension [2, 3]. The fibrous filler is took inspiration from the observation of peripheral nerve system ECM, *in vivo,* which facilitates neurite and cellular migration. According to our previous study, the migration rate of nerve cell cultured on poly(lactic-co-glycolic acid) (PLGA) microfiber is 1.6-fold comparing with flat PLGA membrane [4]. And, the neurite extenuation shows identical direction with microfiber. It has been well know that the micro

pattern directed cell migration and alignment by restriction. 10-30 □m in width of pattern has been proved that is benefice on cell guidance and improving neurite elongation [4-7].

In view of fibrous filler and micro-patterns are beneficial to cell migration and neurite orientation, we develop a hybrid nerve conduit which is microgroove patterned conduit filling with a bunch of microfiber to estimate *in vitro* functionality [4]. 90% of the cells in the hybrid conduit grow in the direction of the designed patterns and microfiber. And, cells fast migration into the interior conduit are observed after 3 days culturing. In this study, we furthered to the hybrid conduit implanted into injured sciatic nerve of mouse to evaluate the bridging efficiency of conduit.

2. Materials and Methods

2.1. Nerve Conduit Fabrication

In this study, two types of nerve conduit were made, Conduit 2.0 and 2.1. The handmade nerve conduit was made by rolling up a microgroove patterned membrane into a PLGA tube (Conduit 2.0). A hybrid-structured nerve conduit which was microgroove patterned membrane wrapped a bunch of microfibers was used as Conduit 2.1.

The fabrication method of a microgroove-patterned membrane and the microfiber were describe in our previous study [4]. The microgroove pattern membrane or microfibers were fabricated by photolithography. In briefly, a cleaned silicon wafer was used as substrate and photoresist coated on the surface by spin-coater. After exposure and development, a micro structure patterned silicon mold was obtained. Poly(dimethylsiloxane) (PDMS) solution was then casted onto silicon mold to transfer micro structures and used as master mold. The PLGA microgroove patterned surface membrane were fabricated by casting 85/15 PLGA solution and dried at room temperature. After de-molding, a both of 30 □m of width and spacing and 2 □m of depth of micro-groove patterned membrane was obtained. Then, PLGA solution dropped into microfiber patterned PDMS mold and scratched off extra solution to dry at room temperature. A bunch of 135 □m of line width, 30 □m of line spacing and 1 cm of length of PLGA microfiber was obtained by carefully de-molding. Each conduit was made into a tube with 1 mm of diameter and 5 mm of length.

2.2. Animal Surgery: Sciatic Nerve Injury and Conduit Implantation

The detail animal surgery procedure was described in our pervious study [6]. In briefly, 8-10 weeks old of FVB mice which maintained in the National Health Research Institutes Animal Canter were used in this study. Mouse was anesthetized by 5% isoflurane air inhalation before surgery, then maintained by 2% isoflurane. A 3 mm mouse sciatic nerve segment in the left leg was excised with microscissor to mimic nerve injury. The ultraviolet sterilized handmade conduit was implanted into injury site to connect 3 mm gap. 1 mm of proximal– and distal-end residual nerve were sutured into conduit, then sutured the muscle and skin to close wound. The surgical implantation groups included the microgroove patterned surface conduit with microfiber (Conduit 2.0) and without microfiber (Conduit 2.1). Mice that treated with same surgery but without conduit implanted were used as negative control group. And, mice did not undergo sciatic nerve injury were defined as Sham control group. All animal experimental procedures were approved by the Instructional Animal Care and Use Committee (IACUC) of the National Health Research and followed ethical guidelines.

2.3. Field Emission Scanning Electron Microscopy

After 4 and 8 weeks implantation, the implanted conduit with surrounding muscle was harvested, and immediately immersed into 4% paraformaldehyde for fixing tissue for 24 hours. The tissue was washed with distilled deionized water for three times, then immersed into 70, 80, 95 and 100% ethanol in series for dehydration. The muscle was removed, and the implanted conduit was sliced into several segments to observe the cross section and inner surface. Each segment was coated with 3 nm thickness of platinum film by sputter for conductive. Field emission scanning electron microscope (FE-SEM) in National Chung Hsing University was used for observation.

2.4. Functional Assessments: Rotarod Test and Compound Muscle Action Potential Measurement

2.4.1. Rotarod Test

Rotarod test was performed with an RT series Rosarod Treadmill (SINGA) at 4 and 8 week post implantation. Before formal data collection, each mouse ran on the 10 rpm, 12 rpm and 15 rpm of the rotating rod for 90 second as a pre-test course, separately. For data collection, mice ran at 20 rpm rotating rod for six times. The maximal recording time was 120 s.

2.4.2. Compound Muscle Action Potential Measurement

Compound muscle action potential was measured at 8 week post implantation and analyzed using BIOPAC MP36 and and BIOPAC BSL4.0 software. A 0.22 mm in diameter of stainless electrode was used. The stimulating electrodes were placed at sciatic notch and recording electrodes were place at gastrocnemius muscle. The distance between stimulating and recording electrode was approximately 2 cm. Stimulation voltage was 6 V, stimulus duration was 0.1 ms, and acquisition length was 200 ms. All mice were continually anesthetized by 2% isoflurane air inhalation and kept warm on electric blanket during measurement.

2.5. Statistics

Data were expressed as mean ± standard error of the mean (SEM). Student's t-test was used for comparing two groups and one-way ANOVA was used for comparing multiple groups. Statistical significance was accepted when $p<0.05$.

3. Results and Discussions

When the nerve is damaged and the function is impaired, the synthetic nerve conduit is one of strategy to connect nerve stump and promoting axon sprouting to re-establish nerve function. However, the conduit shows a failed repair on critical size defect. In order to develop an efficient conduit for nerve regeneration, two mechanical cued structures (microgroove pattern and microfiber) were combined forming a hybrid conduit. It had been demonstrated that possessed the characteristics for nerve guidance and cell invasion [4]. Figure 1 (A) and (B) show the appearance of Conduit 2.0, 2.1 and microfiber filled in conduit. Microgroove patterned membrane and microfiber were fabricated form the poly PLGA by photolithography, electroforming and de-molding. A bunch of 125 □m in width microfiber was wrapped with microgroove patterned membrane forming a 1.5-2 mm in inner diameter conduit for implantation as Figure 1 (A). The inner diameter of conduit fit with the size of mouse sciatic nerve (around 1 mm in diameter) to prevent loose or compressing. Microfibers are clearly observed in conduit and parallel with shell as in Figure 1 (B).

Appropriate mechanical properties and biodegradability of the implanted conduit plays a significant role throughout the regenerative process. The conduit with sufficient flexibility avoids compression of nature nerve, but still provide structural support for nerve fiber regeneration [8, 9]. And, a biodegradable material can be naturally absorbed by the body which is not required a second surgery to remove device [9]. PLGA is a FDA approved biomaterial with suitable mechanical properties, inert bioactivity, and biodegradable [10]. The biodegrade property of Conduit 2.0 were tested in vitro in our previous study, the microfibers were fast degraded in 14 days, but only 5.04% (w/w) degradation rate for microgroove patterned PLGA membrane were observed in 21 days [4]. Besides, the PLGA patterned inner wall was presented porosity at first week. Figure 1(C) shows the degraded morphology of Conduit 2.0 in vivo after 4 and 8 weeks implantation. The microgroove patterns were still maintained at inner surface and the thickness of conduit wall did not show significant difference for 8 weeks implantation. The pores were observed at week 8, but not observed at week 4 on cross section of conduit wall. Microfiber were fragmentation and randomly distributed in conduit at week 8. The comparison of the biodegradation property of Conduit 2.0 *in vivo* and *in vitro*, the degradation was slower *in vivo*. This phenomenon was attributed to the both end of conduit was sutured with nerve stumps which reduced the mass transportation with surrounding environment. And, the end of conduit was gradually closed by regenerated tissue leading the mass exchange completely relied on the osmosis of the conduit wall. In order to prevent the structural collapse during regeneration, the conduit wall have to maintained in a suitable thickness and possess with porosity to increase metabolism which has been proved by Ni et al. [5].

Neurite outgrowth and cell migration on conduit can be modulated by mechanical causes of surrounding environment. Figure 1 (D) magnified photo shows the most infiltrated cells tend to migrate along the microfiber aligned direction when the microfiber are not dramatically degradation. However, the fragmentation microfiber by degradation not only disrupt the cell migrating direction on fibre, but also interfere the guiding ability of microgroove patterned on inner wall. Tissue disorderly grow on conduit with fragmented microfiber as show in Figure 1 (C). The Conduit 2.0 for nerve regeneration still need histological analysis to confirm the nerve fiber regrowth.

Figure 2 shows the functional assay after implanting conduit 2.0 and 2.1for 4 and 8 weeks, respectively. In Rotarod test at week 4 result, Conduit 2.0 did not show significant difference with negative group (No implantation group), but did had when compare to Conduit 2.1 (Figure 2 (A)). In Figure 2 (B), both of Conduit 2.0 and 2.1 presented significant difference with negative group for 8 weeks of implantation. The recovery of CMAP were further measured, Conduit 2.0 did not present a superior recovery as expected in Figure 2 (C). But, the Conduit 2.1 was unexpected that presented a superior recovery. It

implied that Conduit 2.0 obstructed the nerve regeneration even though the result of Rotarod test at week 8 was no difference with conduit 2.1, since the individual difference of mice may affected the result. The repair failure of Conduit 2.0 for nerve injury has two primary reasons, the lower mass transportation and the microfiber obstruction. In this study, a lot of microfiber occupies the space reducing mass exchange rate and cell invasion in conduit, no matter on fiber size or numbers. Hence, the fiber size and filled density are need to be reconsideration even though the cell invasion can along microfiber direction and forming a cluster. [11, 12].

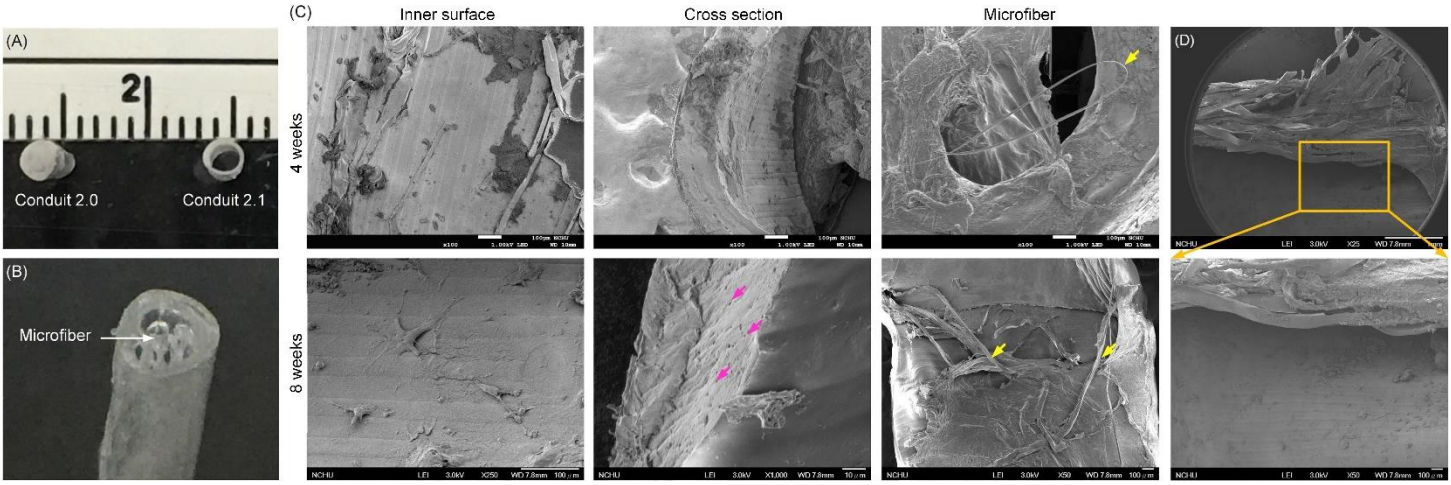

Fig. 1: The appearance of (A) microgroove surface patterned conduit contained with microfibers (Conduit 2.0) and patterned conduit without microfiber (Conduit 2.1). Each conduit was 1.5 – 2 mm in inner diameter. (B) Microfibers were wrapped into microgroove patterned conduit. (C) PLGA degradation SEM images of Conduit 2.0 after implantation for 4 and 8 weeks. Degradation phenomenon was clearly observed at week 8, but all microstructures were still maintained. Pink arrows indicate the pores which are caused from PLGA degradation. Yellow arrows indicate the microfiber location. (D) Microfibers still maintained in orderly arrangement in conduit after 8 weeks implantation. Most migration cells were growth on microfiber and encapsulated the microfiber bunch forming a tissue.

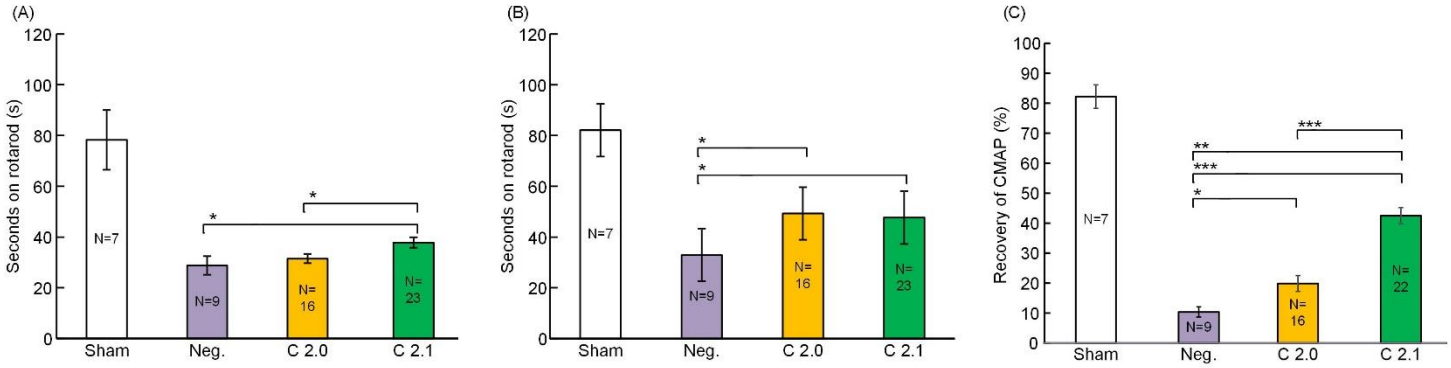

Fig. 2: Results of functional assay. Rotarod test after (A) 4 and (B) 8 weeks implantation. (C) Compound muscle action potential measurement at week 8. Recovery of CMAP was calculated by the measured voltage of injured leg divided to measured voltage of normal leg. Statistical differences are shown as * p<0.05; ** p<0.01; *** p<0.001.

4. Conclusion

A PLGA hybrid conduit which combined two common used mechanical causes, microgroove patterns and microfiber, were developed to evaluate the bridging peripheral nerve injury ability *in vivo*. The degradation rate was slow and the microgroove patterns were still maintained after 8 weeks implantation. The cell migration was along the microfiber orientation and forming a cluster in 8 weeks. Functional assay of CMAP recovery showed that PLGA conduit without microfiber (Conduit 2.1) displayed improved results than PLGA conduit with microfiber (Conduit 2.0) by week 8, even though the Rotarod test did not discern appreciably the two different conduits by week 4 and 8. In the future

studies to modify the mass exchange rate of conduit wall and the microfiber size and filling density of microfiber might be able to improve the nerve regeneration efficiency.

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References

[1] W. Daly, L. Yao, D. Zeugolis, A. Windebank, A. Pandit, "A biomaterials approach to peripheral nerve regeneration: bridging the peripheral nerve gap and enhancing functional recovery," *Journal of the Royal Society Interface*, rsif20110438, 2011.

[2] X. Jiang, R. Mi, A. Hoke, S. Yian, "Nanofibrous nerve conduit-enhanced peripheral nerve regeneration," *Journal of tissue engineering and regenerative medicine*, vol. 8, no. 5, pp. 377-385, 2014.

[3] B. W. Tuft, L. Xu, S. P. White, A. E. Seline, A. M. Erwood, M. R. Hansen, C. A. Guymon, "Neural pathfinding on uni-and multidirectional photopolymerized micropatterns," *ACS applied materials & interfaces*, vol. 6, no.14, pp. 11265-11276, 2014.

[4] S. W. Peng, C. W. Li, I. M. Chiu, G. J. Wang, "Nerve guidance conduit with a hybrid structure of a PLGA microfibrous bundle wrapped in a micro/nanostructured membrane," *International journal of nanomedicine*, vol. 12, pp. 421-432, 2017.

[5] H. C. Ni, T. C. Tseng, J. R. Chen, S. H. Hsu, I. M. Chiu, "Fabrication of bioactive conduits containing the fibroblast growth factor 1 and neural stem cells for peripheral nerve regeneration across a 15 mm critical gap," *Biofabrication*, vol. 5, no. 3, pp. 035010, 2013.

[6] D. C. Lee, J. H. Chen, T. Y. Hsu, L. H. Chang, H. Chang, Y. H. Chi, I. M. Chiu, "Neural stem cells promote nerve regeneration through IL12-induced schwann cell differentiation," *Molecular and Cellular Neuroscience*, vol. 79, pp. 1-11, 2017.

[7] P. A. Wieringa, A. R. G. de Pinho, S. Micera, R. J. A. van Wezel, L. Moroni, "Biomimetic Architectures for Peripheral Nerve Repair: A Review of Biofabrication Strategies," *Advanced healthcare materials*, pp.1701164, 2018.

[8] C. W. Li, Davis, B. J. Shea, H. Sant, B. K. Gale, J. Agarwal, "Optimization of micropatterned poly (lactic-co-glycolic acid) films for enhancing dorsal root ganglion cell orientation and extension," *Neural regeneration research*, vol. 13, no. 1, pp. 105-111, 2018.

[9] A. A. Al-Hamdi, Z. Bukamal, B. C. Leung, "Review Analyzing In Vivo and In Vitro Testing Models on Nerve Conduits of the Peripheral Nervous System," *Iraqi Journal of Medical Sciences*, vol. 12, no. 3, pp. 189-196, 2014.

[10] K. M. Lin, J. Shea, B. K. Gale, H. Sant, P. Larrabee J. Agarwal "Nerve growth factor released from a novel PLGA nerve conduit can improve axon growth," *Journal of Micromechanics and Microengineering*, vol. 26, no. 4, pp. 045016, 2016.

[11] H. S. Ahn, J. Y. Hwang, M. S. Kim, J. Y. Lee, J. W. Kim, H. S. Kim, U. S. Shin, J. C. Knowles, H. W. Kim, J. K. Hyun, "Carbon-nanotube-interfaced glass fiber scaffold for regeneration of transected sciatic nerve," *Acta biomaterialia*, vol. 13, pp. 324-334, 2015.

[12] T. Wu, D. Li, Y. Wang, B. Sun, D. Li, Y. Morsi, E. H. Hany, S. A. D. Salem, X. Mo, "Laminin-coated nerve guidance conduits based on poly (L-lactide-co-glycolide) fibers and yarns for promoting Schwann cells' proliferation and migration," *Journal of Materials Chemistry B*, vol. 5, no. 17, pp. 3186-3194, 2017.