Advanced Drug Delivery Reviews 61 (2009) 1055–1064

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

# The application of nanofibrous scaffolds in neural tissue engineering ☆

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ARTICLE INFO

*Article history:*
Received 25 January 2009
Accepted 16 July 2009
Available online 28 July 2009

*Keywords:*
Nerve regeneration
Central nervous system
Peripheral nervous system
Electrospinning
Self-assembly
Nanofibers

ABSTRACT

The repairing process in the nervous system is complicated and brings great challenges to researchers. Tissue engineering scaffolds provide an alternative approach for neural regeneration. Sub-micron and nano-scale fibrous scaffolds which mimic the topography of natural extracellular matrix (ECM) can be potential scaffold candidates for neural tissue engineering. Two fiber-fabrication methods have been explored in the field of nerve regeneration: electrospinning and self-assembly. Electrospinning produces fibers with diameters ranging from several micrometers to hundreds of nanometers. The fibrous nerve conduits can be introduced at lesion sites by implantation. Self-assembly fibers have diameters of tens of nanometers and can be injected for central nervous system (CNS) injury repair. Both fibrous scaffolds would enhance neurite extension and axon regrowth. These functional nanofibrous scaffolds can serve as powerful tools for neural tissue engineering.



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## 1. Introduction

The regeneration capability of the human adult nervous system is often limited. As a result, patients who have injuries or traumas in the nervous system often suffer from the loss of sensory or motor function, and neuropathic pains. In order to facilitate nerve regeneration, many therapeutic approaches have been attempted. In the peripheral nervous system (PNS), direct end-to-end surgical reconnection is a common method of treatment for nerve transection injuries when the injury gap is small. Nerve autografts are considered as the 'golden standard' for bridging larger nerve defect gaps. However, the shortage of donor grafts, the potential loss of function at donor sites and the requirement of multiple surgeries are among the reasons that constrain the use of autografts. Allografts and xenografts [1–3] are also taken into consideration to substitute autologous nerve grafts. However, these treatments are hampered by immunological rejections and chances of disease transfer. Nerve regeneration in the central nervous

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Nanofibers in Regenerative Medicine & Drug Delivery".

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doi:10.1016/j.addr.2009.07.009

**Table 1**
Nanofibrous scaffolds for nerve regeneration.

| Polymer | Solvent/medium | Diameter | Cells/nerve injury model | Reference |
|---|---|---|---|---|
| A) Electrospun fibrous scaffolds | | | | |
| *In vitro* | | | | |
| Poly(L-lactic acid) (PLLA) | 10 w/v%, chloroform | 500 nm (R/A) | Rat DRG | [38] |
| | 1 wt%, DCM:DMF (7:3 v/v) | (272 ± 77) nm (R) | Neural stem cell line C17.2 | [21] |
| | 1–5 wt%, DCM: DMF (7:3 v/v), | 250 nm (1 wt%, R); 1.5 μm (3 wt%, R); 300 nm (2 wt%, A); 1.5 μm (5 wt%, A) | Neural stem cell line C17.2 | [55] |
| | 10 w/v%, HFP | 100–500 nm (R) | PC12 cells | [43] |
| | 10 w/v%, THF:DMF (1:1 v/v) | (0.51 ± 0.38) μm (R) | Mouse embryonic cortical neurons | [22] |
| | 15 w/v%, DCM:THF (7:3) | (4.3 ± 0.5) μm (R) | Rat Schwann cell (RT4-D6P2T) | [29] |
| Poly($\epsilon$-caprolactone) (PCL) | 16 wt%, DCM: methanol (4:1 v/v) | (1.03 ± 0.03) μm (A); (2.23 ± 0.08) μm (R) | Human Schwann cells | [48] |
| | 20 w/v%, DCM:DMF (4:1 v/v) | 250 nm (A) | Mouse embryonic stem cell | [56] |
| | 9 wt%, chloroform:methanol (75:25 w/w) | (559 ± 300) nm (A) | Chick DRG explants; neurons from dissociated chick DRG; rat Schwann cells; rat OECs | [42] |
| | 15 wt%, chloroform: methanol (75:25 v/v) | (630 ± 40) nm (R) | Rat Schwann cell | [39] |
| | 6 wt%, HFP | (431 ± 118) nm (R) | Neural stem cell line C17.2 | [41] |
| | 12 w/v%, DCM:DMF (1:1 v/v) | (0.95–1.26) μm (R) | Rat Schwann cell line (RT4-D6P2T) | [29] |
| PCL/gelatin | 6 wt%, HFP | PCL/gelatin 50:50: (113 ± 33) nm, PCL/gelatin 70:30: (189 ± 56) nm (R) | Neural stem cell line C17.2 | [41] |
| PCL/collagen | (75:25 wt%), HFP | (541 ± 164) nm (A) | Chick DRG explants; neurons from dissociated chick DRG; rat Schwann cells; rat OECs | [42] |
| PCL/chitosan | (75:25 wt%), HFP and TFA/DCM | (190 ± 26) nm (R) | Rat Schwann cell | [39] |
| Poly(D,L-lactide-co-glycolic acid) (PLGA) | 50 wt%, DMF:THF (1:1 v/v) | 3–5 μm (R/A) | PC12 cells | [95] |
| | 10 w/v%, THF: (1:1 v/v) | (0.76 ± 0.30) μm (R) | Mouse embryonic cortical neurons | [22] |
| Poly-L-lactate (PLA) | 3 wt%, chloroform | (524 ± 305) nm (A) | Rat embryonic DRG | [46] |
| Poly(e-caprolactone-co-ethyl ethylene phosphate) (PCLEEP) | 12 wt%, DCM | (5.01 ± 0.24) μm (A) | PC12 cells | [64] |
| Poly(3-hydroxybutyrate) (PHB) | 14 w/v%, chloroform | (3.7 ± 1.7) μm (A) | Rat Schwann cell line (RT4-D6P2T) | [23] |
| Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(PHBV) | 14 w/v%, chloroform | (2.3 ± 2.1) μm (R) | Rat Schwann cell line (RT4-D6P2T) | [23] |
| Poly (acrylonitrile-co-methylacrylate) (PAN-MA) | 18 w/v%, DMF | (400–600) nm (A) | Rat DRG; rat Schwann cells | [67] |
| Copolymer of methyl methacrylate (MMA) and acrylic acid (AA) (PMMAAA) | 8 wt%, acetone | (450 ± 88) nm (R) | Rat embryonic cortical neural stem cells | [37] |
| Polydioxanone (PDS) | 10 w/v%, HFP | 2–3 μm (R/A) | Rat primary astrocytes; rat embryonic DRG | [47] |
| Polyamide | Commercially available | 180 nm (R) | Rat cerebellar granule neurons. Rat cerebral cortical neurons, rat hippocampal and ventral spinal cord neuronal cultures, rat DRG tissue | [35] |
| Chitosan | 8 wt%, TFA:DCM (75:25 V/V) | (450 ± 48) nm (R) | Rat Schwann cell | [39] |
| *In vivo* | | | | |
| Poly(e-caprolactone-co-ethyl ethylene phosphate) (PCLEEP) | 12 wt%, DCM | (5.08 ± 0.05) μm (A) | Rat sciatic nerve, 15 mm gap | [65] |
| PCL/PLGA | 5.5 wt% PCL and 4 wt% PLGA, chloroform:methanol (3:1 v/v) | 140–500 nm (R) | Rat sciatic nerve, 10 mm gap | [25] |
| Poly($\epsilon$-caprolactone) (PCL) | 15 w/w%, chloroform | 2.5–8 μm (R) | Rat sciatic nerve, 10 mm gap | [25] |
| Poly (acrlonitrile-co-methylacrylate) (PAN-MA) | 18 w/v%, DMF | 400–600 nm (A) | Rat tibial nerve, 17 mm gap | [67] |
| Polyamide | Commercially available | 180 nm (R) | Laminectomy at T8 in rats | [24] |
| Chitosan | 6.4 w/v%, TFA:DCM (4:1 v/v), | (700 ± 502) nm (R) | Rat sciatic nerve, 10 mm gap | [34] |
| B) Self-assembled nanofibers | | | | |
| *In vitro* | | | | |
| Isolucine–lysine–valine–alanine–valine (IKVAV) | Aqueous | 5–8 nm | Murine neural progenitor cells | [57] |
| RAD16-I (AcN-RADARADARADARADA-$COH_2$) | Aqueous | Information not provided | Postnatal rat hippocampal neural cells | [81] |
| RADA16 (Ac-ARDARADARADARADA-$COHN_2$) | Aqueous | 10 nm | Mouse neural stem cells | [75] |
| Synthesized peptide amphiphile (PA) containing RGD (arginine–glycine–aspartic acid) | Aqueous | 7.6 ± 1 nm | Rat mesenchymal stem cells from femur bone shaft | [82] |
| Arginine–alanine–aspartate RAD 16-I, and RAD 16-II | Aqueous | 10–20 nm | PC12 cells; mouse cerebellar granule neurons; mouse hippocampal neurons; rat hippocampal neurons | [80] |
| *In vivo* | | | | |
| Arginine–alanine–aspartate–alanine (RADA 16-I) | Aqueous | 10 nm | • Knife wound in superior colliculus in hamsters<br>• Optic tract transection at brachium of mouse spinal cord | [92] |

**Table 1** (*continued*)

| Polymer | Solvent/medium | Diameter | Cells/nerve injury model | Reference |
|---|---|---|---|---|
| *In vivo* | | | | |
| RADA 16-I | Aqueous | 10 nm | 1 mm dorsal column tissue removal between C6 and C7 in rats | [88] |
| Isolucine–lysine–valine–alanine–valine (IKVAV) | Aqueous | Information not provided | Spinal cord compression at T10 of mouse | [96] |

Legend: A: aligned, non-woven fibrous mesh; DCM: dichloromethane; DMF: dimethylformamide; DRG: dorsal root ganglia; HFP: 1,1,1,3,3,3 hexafluoroisopropanol; OEC: olfactory ensheathing cells R: random, non-woven fibrous mesh; RAD16-I: + − + − + − + − AcN-RADARADARADARADA-CNH2; RAD16-II: + + − − + + − − AcN-RARADADARARADADA-CNH2; THF: tetrahydrofuran; TFA: trifluoroacetic acid.

system (CNS) is even more challenging, with the inhibitory environment formed after injury in the CNS often restricting nerve regeneration [4]. Current methods of treatment are often not effective enough to restore nervous function in the CNS.

Due to the above reasons, tissue engineered scaffolds may serve as an alternative choice for implantation to facilitate neural repair. The physical and chemical properties of artificial grafts can be tailored based on applications. Biodegradable and biocompatible materials are often used to reduce the occurrence of immune responses. By controlling scaffold morphology, architecture and components, suitable topographical and biochemical cues may be provided to promote tissue regeneration. These artificial scaffolds can also be modified to provide a permissive substrate for axons to penetrate the injured area in CNS tissue engineering [5].

A common approach in tissue engineering is to mimic the architecture of the natural extracellular matrix (ECM). The ECM plays an important role in regulating cellular behaviors by influencing cells with biochemical signals and topographical cues [6,7]. It has two main components: polysaccharides and fibrous proteins. As such, nanofibrous constructs have been used extensively as potential tissue engineering platforms. It is generally hypothesized that a close imitation of the ECM will provide a more conducive environment for cellular functions ranging from adhesion, migration, proliferation to differentiation [8]. Meanwhile, nanofibers exhibit extremely high surface area-to-volume ratio. This property facilitates the release of biochemicals, including proteins, drugs, and nucleic acids [9–11], which may be delivered by the fibers. Additionally, the large surface increases the contact area between cells and the fibers, thereby enhancing chemical uptake by cells. Therefore, nanofibrous scaffolds may also be used as a potential drug carrier for chemical therapy.

Many approaches have been developed to produce nanofibers. In this paper, we will review two widely-studied fiber-fabrication methods as applied to neural tissue engineering, namely electrospinning and self-assembly. We will also discuss in detail the respective outcomes of using such nanofibers for nerve repair and regeneration as evaluated by *in vitro* and *in vivo* studies.

## 2. Electrospun fibrous scaffolds in nerve tissue engineering

Electrospinning is a simple method of producing continuous fibers with diameters within the micron to sub-micron regime [12–14]. The flexibility of the technique is evident from the ease of forming fibers out of a wide range of materials including natural and synthetic polymers, composites and ceramics [15,16]. Like conventional fiber spinning processes, parameters such as polymer solution concentration and viscosity, and polymer solution dispense rate may be controlled during fabrication to alter the dimensions of the fibers. In addition, because of the nature of electrospinning, electrical field strength and field pattern may be varied to manipulate the architecture and morphology of the resulting scaffold. For a comprehensive review on the electrospinning process and approaches to control scaffold architecture, one may refer to the works by Chew et al. [12] and Murugan et al. [17].

Due to the versatility of the electrospinning technique, electrospun scaffolds have found applications in various areas of tissue engineering ranging from cardiovascular [18] to musculoskeletal tissue engineering [19] and stem cell engineering [20]. The large surface area-to-volume ratio property of these scaffolds ensures that any incorporated bioactive agents are released efficiently and that the contact between cells and these biochemicals is maximized.

The feasibility of using electrospun fibrous scaffolds as substrates for neural tissue engineering has also been investigated by several research groups [21–23]. Comparisons between substrates made out of polymer films versus electrospun fibers have been tested both *in vitro* and *in vivo* to demonstrate the efficacy of these fibers in enhancing nerve regeneration. *In vivo*, axonal extension within electrospun fibrous nerve conduits and partial functional reconnection have been demonstrated [24,25]. Altogether, these findings indicated that electrospun fibers are potential candidates for nerve repair applications [26,27]. A summary of electrospun fibrous scaffolds used in nerve regeneration studies is listed in Table 1.

### 2.1. Effects of electrospun materials on nerve regeneration

Material choice plays a vital role in ensuring the success of neural tissue engineering strategies. Appropriate material degradation rate and mechanical properties help minimize inflammatory response and prevent nerve compression situations, while providing the required support and guidance to regenerating axons. With these requirements in mind, the search for the suitable scaffolding material remains and many have been attempted via electrospinning. These materials may be broadly categorized into three groups: 1) synthetic materials; 2) natural materials; and 3) biosynthetic materials.

#### 2.1.1. Electrospun biocompatible synthetic materials

Biocompatible synthetic materials are attractive for constructing neural tissue engineering scaffolds because of the ease in tailoring the degradation rate and mechanical properties of these materials to suit the applications. Polyesters such as poly(glycolic acid) (PGA), poly (L-lactic acid) (PLLA), poly(caprolactone) (PCL), poly(3-hydroxybutyrate) (PHB) and their copolymers have been extensively investigated as electrospun scaffolds applied to neural tissue engineering (see Table 1). Polyesters can be degraded by hydrolysis of the ester bond leading to degradation products that can be resorbed through metabolic pathways, causing minimal toxicity to the hosts [28]. Other synthetic polymers such as polydioxanone (PDS), poly (acrylonitrile-co-methylacrylate) (PAN-MA) have also been reported as materials for electrospun fibers to induce nerve growth. In general, these synthetic materials support neural cell growth, but their ability in terms of supporting cell proliferation and viability differs due to their distinct properties.

Sangsanoh et al. [29] studied the cytocompatibility of several biodegradable polymeric electrospun fibers and solution-cast films by culturing rat Schwann cells on these platforms. After 24 h, the rank of cell viability on various substrates was: PCL film>TCPS>PCL fibers> PLLA fibers>PHBV film>chitosan (CS) fibers≈CS film≈PLLA film> PHB film>PHBV fibers>PHB fibers. The rank, however, was different at day 3: TCPS>PHBV film>PLLA film>PCL film>PLLA fibers>PHB film≈PCL fibers>CS fibers>CS film>PHB fibers>PHBV fibers. While it was clear that scaffold architecture and chemistry affect Schwann

cell viability, the exact relationship between these factors and Schwann cell behavior could not be elucidated from the study due to the lack of obvious experimental trends. An additional size effect was also present due to the different fiber diameters of different electrospun materials, which varied from 130 nm to 3.7 μm. More in-depth comparison studies are thus required in order to understand the mechanisms of how material property may affect neural cell behaviors.

#### 2.1.2. Electrospun natural materials

Natural materials may share similar properties with the soft tissues which they are replacing, such as similar mechanical strength, physical properties and biomolecular recognition sites [30]. Collagen, gelatin, laminin and chitosan [31–33] are among the examples of electrospun natural materials that have been used for nerve reconstruction. Wang et al. [27,34] developed chitosan micro/nanofiber mesh tubes for treatment of a 10 mm rat sciatic nerve gap. It was observed that sensory function begun to recover as early as 8 weeks after implantation of the nano/microfiber mesh tubes. Regenerating axons also elongated through the lumen of the chitosan tubes. In spite of the regeneration obtained in these fibrous scaffolds, the weak mechanical properties of natural electrospun constructs may limit their application as nerve guide conduits. In order to maintain structural integrity during both operation and post-operation periods, sufficient mechanical strength is required of a nerve conduit. As evident from Wang's study, tube collapse may occur when the degree of acetylation of chitosan is lower than 93%. Meanwhile, the use of natural materials is also jeopardized by its high cost, possible immunogenicity and batch-to-batch variability.

#### 2.1.3. Electrospun biosynthetic materials

Biosynthetic materials serve to combine the merits of both synthetic and natural materials, with improved mechanical properties over natural polymers and enhanced biocompatibility over synthetic ones. These unique properties can be achieved by covalent binding or blending synthetic and natural polymers, or surface adbsorption.

Ahmed et al. [35] surface-modified polyamide nanofibers by covalent attachment of fibronectin type III repeat D of human tenascin C, D5 and extended version D5'. These peptides were identified to promote neurite outgrowth for cerebellar granule neurons [36]. After covalent modification with these neuroactive peptides, neuronal cell attachment, neurite generation and neurite extension were significantly enhanced as compared to plain electrospun polyamide fibrous scaffolds. Collagen was also conjugated onto a copolymer of methyl methacrylate (MMA) and arylic acid (AA) (PMMAAA) electrospun nanofibers by Li et al. [37]. The collagen content ranged from 0% to 5.7% in the electrospun fibers. It was observed that at Day 2 and Day 4, the neurite length of cortical neural stem cells increased in accordance with collagen content. At the same time, cell viability was enhanced with respect to the increase in collagen content at Day 4. This indicated that the collagen-conjugated electrospun fibers could improve the attachment and viability of the cultured neural stem cells. The advantages of biosynthetic materials were also demonstrated by Patel et al. [38] through the immobilization of laminin onto PLLA nanofibers for the culture of rat dorsal root ganglia (DRG) tissues. After culturing for 6 days *in vitro*, cells on laminin-conjugated PLLA fibers had significantly longer neurite length as compared to cells grown on plain PLLA fibers.

Due to the uniqueness of electrospinning, an even simpler way of producing biosynthetic scaffolds is to prepare a polymer blend as the working material during the fabrication process. By choosing suitable solvents, synthetic and natural materials may be dissolved into the same medium and uniform mixtures can be obtained for electrospinning. Prabhakaran et al. [39] blended PCL with chitosan to produce electrospun nanofibers. These blended scaffolds (PCL/CS) had a narrow distribution of fiber diameter and improved hydrophilicity as compared to PCL fibers. With the appropriate hydrophilicity, cell adhesion and spreading became more rapid and more effective [40]. Moreover, the tensile strength and ultimate strain of the PCL/CS scaffolds increased in comparison with chitosan fibers. Their study demonstrated that the proliferation rate of rat Schwann cells on PCL/CS nanofibers was significantly higher than that on PCL nanofibers. Biosynthetic electrospun fibers were also fabricated by Ghasemi-Mobarakeh et al. [41]. By comparing PCL/gelatin electrospun scaffolds at 100:0, 70:30 and 50:50 weight ratios, the presence of gelatin improved the hydrophilicity of PCL scaffolds. C17.2 neural stem cells showed longer neurite length and higher proliferation rate on 70:30 PCL/gelatin fibers in comparison with PCL constructs. Although the inclusion of gelatin positively influenced neural stem cell behavior, there appeared to be a limit of ~50 wt.% gelatin that one may include into PCL fibers, as indicated by this study. 50:50 PCL/gelatin fibrous constructs, while demonstrating higher hydrophilicity, also possessed loose and weak fibrous structures and enhanced degradation rate that may not be favorable for nerve regeneration. Schnell et al. [42] compared PCL and PCL/collagen nanofibers as nerve regeneration scaffolds. They found that both kinds of scaffolds were favorable substrates for neurite outgrowth and glial migration from DRG explants. However, Schwann cell migration, neurite orientation and process formation of Schwann cells, fibroblasts and olfactory ensheathing cells improved on PCL/collagen fibers.

Finally, the effects of the mode of biopolymer inclusion to synthetic electrospun fibers on neural cell behavior were evaluated by Koh et al. [43]. The authors studied three different methods of incorporating laminin into PLLA fibers — covalent binding, physical adsorption and blended electrospinning. In terms of supporting PC12 cell proliferation and enhancing neurite outgrowth, blended electrospinning was the most facile and efficient method as compared to the other two modification approaches.

In all studies, it appears that the presence of biopolymers, although drastically changing the degradation rate and mechanical performance of synthetic polymeric scaffolds, significantly helps in promoting neural cell proliferation and neuron extension. Such materials may find useful applications in promoting nerve regeneration *in vivo*.

### 2.2. In vitro studies

#### 2.2.1. Effects of electrospun scaffold architecture on nerve regeneration

*2.2.1.1. Neurons and axonal outgrowth.* Neurite outgrowth and cell migration are indispensible processes for nerve repair. Guided axonal extension towards designated targets to reform synaptic connections can help in the restoration of nerve functions. The migrated glial cells to the injured sites can secrete neurotrophic factors and support regenerated neurons to help nerve regeneration. Accordingly, the ability to enhance neurite outgrowth, direct axon extension, and facilitate cell migration is an essential factor to be included during the design of tissue engineering scaffolds for nerve repair. While micro- and nano-patterned substrates have been extensively studied to understand the effects of contact guidance on neural cell behavior and function [44,45], electrospun scaffolds with aligned fibers are also gaining importance for their promise in providing topographical cues for nerve regeneration and as direct implantable devices.

Compared to randomly-oriented fibers, aligned fibers are able to promote neurite outgrowth (Fig. 1). DRG cultured on PLLA randomly-oriented nanofibers showed no neurite outgrowth after 6 days. In contrast, significant extension of neurites from DRG explants was observed on aligned fibers [38]. In addition, many studies have proved that neurons cultured on aligned electrospun fibers have longer neurite lengths than those on random fibers [42,46,47].

The aligned fibrous scaffolds serve as contact guidance for directing neurite orientation and cell alignment (Fig. 2). It is found that neurites emanated radially from DRG tissues when cultured on

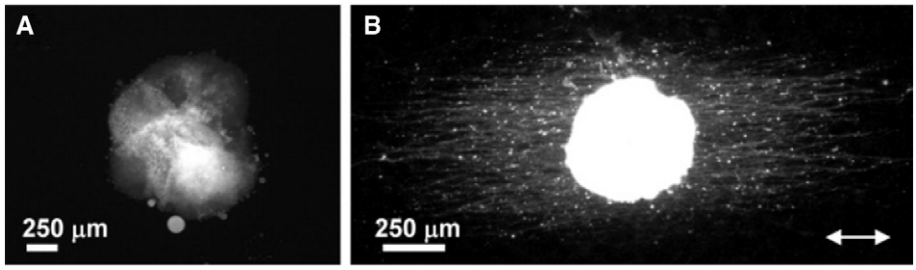


**Fig. 1.** Neurite outgrowth from DRG tissue on PLLA nanofibers. DRG was cultured on PLLA (A) randomly-oriented and (B) aligned nanofibers for 6 days *in vitro*. The neurofilaments staining indicated significant neurite outgrowth from DRG cultured on PLLA aligned nanofibers. Adapted with permission from American Chemical Society [38].

randomly-oriented electrospun fibers, generating a round appearance. In contrast, on aligned fibrous substrates neurites grew in a radial manner initially when they emanated out from DRGs, but would follow the orientation of the fibers as they continued to grow. In addition, neurites that grew in the direction of the underlying fibers had a faster growth rate than those perpendicular to the aligned fiber axis or those grown on randomly-oriented fibers. The same growth behavior was observed in astrocytes. On randomly-oriented fibers, however, astrocytes were positioned more haphazardly. A branched net shape structure was observed due to the lack of specificity in the direction of projections by the processes of the astrocytes. In contrast, astrocytic processes were well aligned, following the orientation of the fiber axis when cultured on aligned fibrous substrates [47].

Different neurite outgrowth results were observed in both studies discussed above [38,47]. It is possible that the variations in scaffold properties, cell culture conditions and duration influenced neurite outgrowth. Scaffold properties such as material, fiber diameters, and scaffold porosity can affect cellular behavior. In work by Petal et al., PLLA was used to fabricate electrospun fibers of diameter of 500 nm while in the latter case, PDS was chosen and the fiber diameter ranged between 2 and 3 μm. Meanwhile, cell culture condition also plays a very important role in affecting neurite outgrowth. Different types of culture medium used in these two studies could strongly affect cell growth. Neural basal medium supplemented medium was used for cell culture in Petal et al.'s work. However, Chow et al. added nerve growth factor to supplement cell growth. Embryonic and postnatal cells harvested for these two studies may also have varied activities in terms of neurite extension. Lastly, in Patel et al.'s work, the cells were cultured for 6 days whilst Chow et al. incubated cells for 10 days which may allow more extensive neurite outgrowth. Altogether, these differences may explain the variations in neurite outgrowth on random fibers in both studies.

Corey et al. [46] also found that ganglia changed their shape as a result of culture on aligned electrospun fibers. They explained that the ganglia deformation in the direction of the fibers was encouraged by the tension, which was generated by neurites following the fiber orientation. This elongated morphological change was also observed for human Schwann cells cultured on micron-sized electrospun fibers (Fig. 3.) [48], indicating that the diameter of the fiber, at least up to 2 μm, is not a crucial factor in influencing cell alignment. The alignment of glial cells would further enhance the rate and extent of neurite outgrowth [49]. Additionally, these morphological alterations may elicit functional changes that will influence cellular phenotypes, ranging from apoptosis to proliferation, cell migration, differentiation, contractility and gene expression [50–52].

*2.2.1.2. Schwann cells.* Schwann cells support peripheral nerve recovery by secreting neurotrophins and producing ECM molecules which elicit axonal regrowth [53]. The migration and formation of aligned Schwann cells to form bands of Büngner have to precede the infiltration of axons following nerve injuries. However, Schwann cell migration is often the rate-limiting step. Therefore, one of the approaches to enhance peripheral nerve recovery is to imitate the formation of bands of Büngner *in vitro*.

The possibility of guiding Schwann cell migration by aligned fibers has been studied *in vitro* by Schnell et al. [42]. By culturing DRG explants on blended PCL/collagen (4:1 w/w) aligned fibrous scaffolds, rat Schwann cells were observed to migrate away from the DRG along the direction of the aligned fibers. After 7 days of culture, more than 1 mm distance of migration on PCL/collagen aligned fibers was seen. Although aligned fibrous scaffolds could induce a more obvious alignment of the cells, random fibrous substrates also provide contact guidance to some extent, as the cells may stretch the cytoskeleton across multiple fibers and follow the fiber orientation. Chew et al. [48] found both randomly-oriented and aligned electrospun PCL fibers to decrease the expression of neurotrophin and neurotrophic receptors of human Schwann cells after 7 days of *in vitro* culture, indicating a more mature phenotype adopted by cells cultured on fibrous

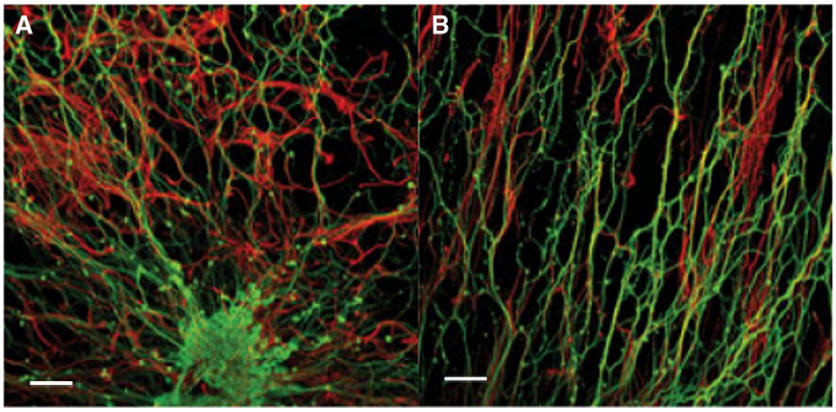


**Fig. 2.** The effect of electrospun fiber orientation on neurite alignment. Immunostaining of astrocytes (red) and DRG neurites (green) on (A) random polydioxanone (PDS) nanofibers and (B) aligned PDS electrospun fibers after 20 days *in vitro* culture. Scale bar: 100 μm. Adapted with permission from Cambridge University Press [47]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

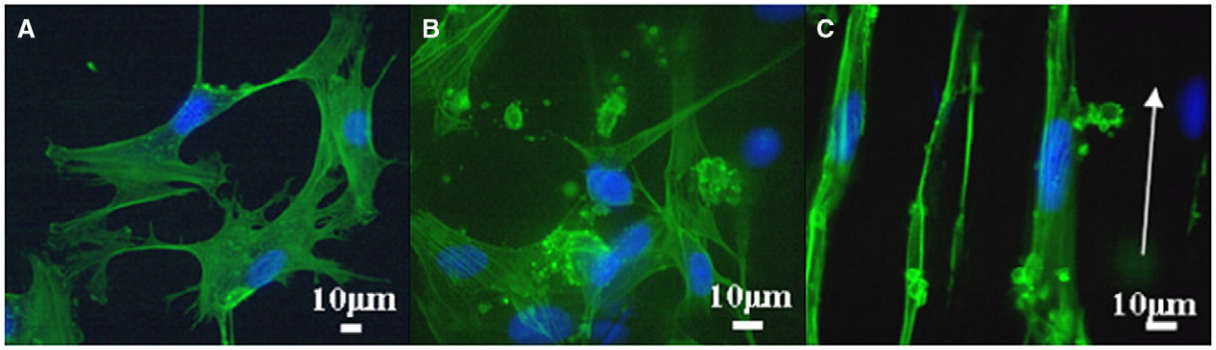


**Fig. 3.** The effect of contact guidance of aligned electrospun fibers on human Schwann cell. Human Schwann cells were cultured for 3 days on (A) PCL film, (B) PCL random fibers and (C) PCL aligned fibers. Adapted with permission from Elsevier [48].

substrates. Aligned fibrous scaffolds, however, enhanced Schwann cell maturation more than randomly-orientated fibers, as more substantial up-regulation of the gene expression of myelin specific marker, P0, was observed in cells grown on aligned fibers.

*2.2.1.3. Stem cells.* Stem cells have recently been considered and studied as one of the cell-based therapies in neural tissue engineering. Stem cells are able to differentiate into various cell types. These stem cells can be transplanted to the injured sites in the nervous system, replacing missing cells and activating endogenous cells to perform self-repair [54].

In an effort to understand stem cell–substrate interactions, various cell types have been analyzed on electrospun scaffolds. The differentiation rates of neural stem cell C17.2 cultured on PLLA electrospun fibers were found to be similar on random and aligned fibers, but were dependent on fiber diameters [55]. By counting the number of cells that exhibited elongated shape under the laser scanning confocal microscope, the differentiation rate of C17.2 after 2 days *in vitro* was quantified. On nanofibers with average diameter of 300 nm, cells demonstrated a differentiation rate of 80%. In contrast, the differentiation rate of C17.2 on micron-sized fibers with average diameter of 1.5 μm was only 40%.

The differentiation of mouse embryonic stem (ES) cells on the electrospun nanofibers was also explored [56]. Mouse ES cells were cultured in the presence of retinoic acid, which could induce ES cell differentiation along the neural lineage. Both random and aligned fibrous substrates were supportive of stem cell neural differentiation into neurons, oligodendrocytes and astrocytes. Interestingly, the aligned nanofibers discouraged the differentiation of ES cells into astrocytes. Therefore, aligned fibers might be a potential therapeutic approach for CNS injuries, as astrocytes have been found to be the main reason for glial scar formation. A similar finding was also reported with self-assembled nanofibers minimizing astrocytic differentiation [57]. Altogether, the use of nanofibrous scaffolds as stem cell culture platforms may be promising in CNS repair, supplying neurons and at the same time, inhibiting glial scar formation.

In general, multiple works have shown that cells do respond to the unique fibrous architecture of electrospun scaffolds. In most studies, cellular response evaluation remains restricted to understanding cell proliferation and morphological changes with respect to topographical signals. Only few recent studies have attempted to elucidate in detail the functional changes of neural cells in terms of gene expression and cellular differentiation [48,57]. More analysis is, therefore, required to ensure that the observations of cell shape changes do correspond to useful cell functions that will indeed aid nerve regeneration *in vivo*. At the same time, deeper understanding of the mechanisms, such as cell signaling processes, behind the corresponding cellular responses to topographic stimuli will also help in facilitating precise control of cellular functions and stem cell differentiation using electrospun fibers.

*2.2.2. Effects of controlled release of biochemicals from electrospun scaffolds on nerve regeneration*

Neurotrophic factors have profound effects on neural development and functions. These factors are usually expressed in large amounts in the presence of nerve injuries and play crucial roles in supporting neural cell survival and axon regrowth [58,59]. Multiple delivery methods have been extensively investigated to introduce these factors to the injury sites in both CNS and PNS, such as in the form of microspheres [60,61] and polymer matrices [62,63]. Electrospun nanofibers may serve as a promising delivery vehicle due to its simultaneous ability to provide scaffolding function and contact guidance that may be necessary for nerve regeneration.

Using a copolymer of caprolactone and ethyl ethylene phosphate, PCLEEP, Chew et al. [64] demonstrated that by directly blending human β nerve growth factor (NGF) into PCLEEP polymer solution during electrospinning, protein-encapsulated electrospun fibers could be obtained. The encapsulated protein was found to be dispersed uniformly throughout the fibers in aggregate form due to phase separation. A sustained release of bioactive NGF from aligned electrospun fibers was obtained for at least 3 months *in vitro*. Following that, the efficacy of such biofunctional electrospun scaffolds was evaluated in an *in vivo* study by the same group [65]. By using human glial cell-derived neurotrophic factor (GDNF)-encapsulated aligned electrospun fibrous conduits, sciatic nerve regeneration in a rat model was significantly enhanced. The total number of myelinated axons, area of regenerated nerve cross-section and G ratio value were significantly larger than the control groups comprising of plain electrospun scaffolds and PCLEEP films. Detailed information on the study is discussed in the next section. Altogether, the ability of incorporating growth factor signaling and contact guidance within an electrospun scaffold demonstrates the uniqueness of this fabrication technique and its potential in enhancing nerve regeneration.

In a separate study, surface modification of electrospun scaffolds with growth factors was adopted instead. Patel et al. [38] explored the effects of immobilizing basic fibroblast growth factor (bFGF) onto nanofibers on DRG neurite extension *in vitro*. As compared to plain aligned electrospun fibers and bFGF-immobilized randomly-oriented fibers, the highest neurite outgrowth was achieved on a combination of aligned fibers with either immobilized bFGF or soluble bFGF introduced directly into the cell culture medium. In addition, the conjugated biofunctional fibers presented several advantages over delivering bFGF in soluble manner. First, only a small amount of bFGF was required to achieve similar effects as soluble bFGF in medium. Secondly, the electrospun fibrous scaffolds can act as a delivery vehicle for specific targets, without inducing systemic effects.

The studies highlighted above clearly demonstrated the efficacy of electrospun nanofibrous scaffolds as biofunctional substrates that combine topographical and biochemical signaling that may be vital for enhancing neural tissue regeneration.

### 2.3. In vivo studies

Random electrospun nanofibers have been applied for the regeneration of both CNS and PNS injuries. Meiners et al. [24] investigated the feasibility of using polyamide nanofibers for over-hemisection spinal cord injury in rats. A laminectomy was made at thoracic level 8 spinal vertebrae and the nanofibrous fabric was crumpled into the lesion site. After 3 weeks, immunohistochemical staining showed axonal growth at injury sites and the axons extended onto the nanofibers. Unfortunately, this implantation method led to the self-folding of the scaffolds, and the regenerated axons followed the surface contours of the random folds, which were not targeting the distal stump. Certain orientations of the scaffolds should, therefore, be considered for implantation, such as employing multilayered tubular structures [66].

Promising results of using random electrospun fibers for PNS injuries have also been reported. Panseri et al. [25] fabricated PLGA/PCL electrospun fibrous tubes to bridge a 10 mm nerve gap in the rat sciatic nerve. After 4 months, regenerated tissues filled the inner lumen of the fibrous tubes and bridged the nerve gaps. Neurite outgrowth was found along the longitudinal conduit axis. At the same time, myelination and collagen IV deposition were detected in concurrence with regenerated axons. Von Frey test was carried out to evaluate sensory function recovery after implantation every 2 weeks. Rats were placed on a metal mesh floor and a series of Von Frey hair was applied to the plantar surface of the hind paw. Brisk withdrawals or paw lickings were accounted as positive responses. A decreasing trend of sensitivity threshold was observed in nerve conduit-treated animals as the test progressed, whereas no positive response to stimuli was seen in the untreated injured animal group. Electromyography tests revealed that 70.6% of the fibrous tube-treated rats showed an initial reinnervation in plantar muscles, while no CMAP (compound muscle action potential) was present in non-treated animals. However, both mean values of CMAP and MCV (motor conduction velocity) were lower in treated animals as compared to healthy animals, indicating a relatively early phase of nerve regeneration.

Aligned fibers are able to enhance neurite outgrowth and direct neurite extension *in vitro*. In order to evaluate the effectiveness of such scaffolds in enhancing nerve regeneration and functional recovery via contact guidance, several *in vivo* studies have been carried out.

Kim et al. [67] stacked both randomly-oriented and aligned electrospun fiber sheets into polysulfone to form guidance conduits for nerve repair in a 17 mm rat sciatic nerve transection model. After 16 weeks in aligned fibrous constructs, the number of axons per cross-section was significantly higher than in conduits comprising randomly-oriented fibers. In aligned fibrous conduits, the regenerated axons were myelinated and extended into the distal end. Behavioral assessment by grid walking test and electrophysiology test was carried out after 16 weeks to measure functional recovery. When provided the same level of stimulation, similar compound action potentials (CAP) and muscle contractions were observed in regenerated nerves treated with autografts and aligned constructs. However, in animals where the nerves are bridged by random constructs, no CAP was detected. Grid walking tests demonstrated that in animals that received autografts and aligned constructs, significantly fewer foot slips were recorded than in random construct- or saline filled polymeric construct-treated animals. These findings showed that aligned fibrous constructs had comparable performance in reducing the functional deficits after nerve injury as autografts. It is believed that contact guidance played an indispensible role in the regeneration process observed in the study. The stacked structure of the aligned fiber conduit was three-dimensional and therefore maximized the topographic directional cues.

Using an alternative approach of rolling up a sheet of aligned electrospun fibers in different directions, hollow nerve guide conduits were obtained by Chew et al. [65]. These nerve conduits comprised hollow centers with aligned fibers oriented either longitudinally or circumferentially along the inner surface of the conduits. After 3 months of implantation to bridge a 15 mm gap in the rat sciatic nerve, both fibrous conduits had higher numbers of myelinated axons and larger nerve cross-sectional area as compared to plain polymer tubes without electrospun fibers. Interestingly, electrospun fibers of both longitudinal and circumferential orientation resulted in similar extent of nerve regeneration and functional recovery. In this case, it seems that contact guidance was not the reason for promoting axon regeneration but rather the increased surface area for cell adhesion.

Although many *in vitro* studies have verified that aligned fibers are able to drastically enhance neurite outgrowth rate and direct axon extension, only few *in vivo* studies have actually been carried out to verify the usefulness of such contact guidance on nerve regeneration and functional recovery. Therefore, the concrete conclusion of aligned fibers in enhancing nerve repair *in vivo* by either contact guiding cues or enhanced cell attachment area cannot be derived until more research has been implemented.

While the efficacy of electrospun fibers in nerve regeneration is clearly demonstrated in the above studies, it remains difficult for direct comparisons of the experimental outcomes. This is largely due to the variation in test methods used. In terms of experimental approach, differences in injury gap sizes adopted and the length of time post-surgery prior to nerve recovery assays exist. In terms of nerve regeneration and functional recovery assays utilized, immunohistochemical morphological evaluation, functional assays involving grid walking/foot slip tests and measurements of compound muscle action potentials at various different locations of the regenerated nerve and muscles may all contribute to different experimental results. More conclusive outcomes can only be derived once common test methods are adopted in unison for fair comparisons between studies. Nonetheless, in general, after nerve injury treatment, myelination of regenerated nerves is anticipated to occur at earlier time points before functional recovery is observed. Success in nerve myelination and functional recovery is also anticipated, across small lesion gaps such as 10 mm and below in the rat model. From the studies above, it also appears that functional recovery can be observed as early as 3 months post-implantation of fibrous scaffolds. Additionally, in order to truly demonstrate the efficacy of the electrospun fibrous scaffolds, critical defect gap sizes of at least 15 mm should be considered.

## 3. Self-assembled nanofibrous scaffolds in neural tissue engineering

Self-assembly as another frequently-used technique to fabricate nanofibers, is the spontaneous organization of molecules and components into patterns or structures without human intervention [68]. Self-assembling process is mediated by non-covalent bonds, such as van der Waals forces, hydrogen bonds, and electrostatic forces [69]. A lot of studies showed that a broad range of proteins and peptides can produce very stable and well ordered nanofiber structures with remarkable regularity [70–73]. Moreover, these peptide molecules can break down into natural L-amino acids which are nontoxic and could potentially be used by nearby cells for growth and repair [74]. The diameters of self-assembled nanofibers are at least one to two orders of magnitude smaller than typical electrospun fibers. Therefore, it is believed that self-assembled nanofibrous scaffolds can provide cells a true three-dimensional (3-D) microenvironment that is similar to the natural extracellular matrix [75]. All these benefits make it very promising for using functionally designed self-assembled nanofibers

in regenerative medicine [76–79] and in neural regeneration studies. A summary of self-assembled nanofibers used in both *in vitro* and *in vivo* studies of neural tissue engineering is listed in Table 1.

### 3.1. In vitro studies

Arginine–Alanine–Aspartate (RAD)16-I and RAD16-II are the most commonly used peptides in self-assembled peptide nanofibrous scaffolds (SAPNSs) for neuronal cell culture [57,75,80–82]. Several types of neuronal cells have been grown on RAD16 self-assembling peptide scaffolds (sapeptide scaffolds) by Holmes et al., including rat PC12 cells, primary cells isolated from 7-day old mice cerebellum and hippocampus, and fresh neuronal cells prepared from neonatal rats [80]. In the case of PC12 cells, extensive neurite outgrowth along the contours of the sapeptide scaffolds was observed 24 h after NGF treatment. Neurite extensions were also found in primary neuronal cell cultures on sapeptide scaffolds. Besides supporting neuronal cell growth, sapeptide scaffolds also promoted the formation of functional synapses in rat hippocampal neurons as evaluated by positive FM1-43 staining. In an independent study, a 3-D cell entrapment system established using RAD16-I sapeptide scaffolds to culture primary rat hippocampal cells was reported by Semino et al. [81]. BrdU and GFAP staining showed that glial cells and neurons migrated and proliferated in the sapeptide scaffolds in 1-week cultures. Positive nestin signal detected on the interface between the hippocampal tissue slice and the sapeptide scaffolds suggested the presence of neuroprogenitor cells. Cell migration into the scaffolds after 3 days culture was also much higher as compared to that on a control comprising a Millipore membrane (30-mm culture plate insert with 0.4-μm pore), which was used as a basic hippocampal organotypic slice culture technique. These results suggested that the sapeptide scaffolds may be an attractive substrate for supporting neural tissue growth and regeneration.

In another study, RAD16 sapeptide scaffolds with different functional motifs, including RGD and laminin-derived motifs, GFLGFPT and BMHP, were synthesized for culturing adult mouse neural stem cells [75]. Cell differentiation was evaluated at Day 7, and the results showed higher numbers of cells expressing neuronal marker, β-Tubulin, and astrocytic marker, GFAP, on RAD16-BMHP1 and RAD16-BMHP2 sapeptide scaffolds. On the other hand, more cells remained in the undifferentiated state when cultured on pure RAD16 sapeptide scaffolds. The study clearly suggested that sapeptide scaffolds combining functional motifs could be promising tools for 3-D neural cell culture and nerve repair.

Isoleucine–lysine–valine–alanine–valine (IKVAV), which is known to promote and direct neurite outgrowth [83,84], is another commonly used sapeptide in SAPNSs for neural regeneration studies. Silva et al. [57] cultured murine neural progenitor cells (NPCs) on IKVAV sapeptide scaffolds to study cellular differentiation *in vitro*. At Day 1 and Day 7, the scaffolds induced a selective differentiation in the cells by promoting differentiation into neurons, while suppressing astrocytic differentiation. The IKVAV sapeptide scaffolds also promoted greater and faster differentiation of the progenitor cells into neurons relative to poly(D-Lysine)- or laminin-coated substrates [85–87].

### 3.2. In vivo studies

In addition to *in vitro* experiments, animal models have also been used to investigate the possibilities of using SAPNSs for treatment of CNS injuries. Guo et al. [88] transplanted RAD16-I sapeptide scaffolds cultured with adult rat Schwann cells and embryonic NPCs isolated from hippocampus into adult rats with spinal cord dorsal column transection. After 6 weeks transplantation, the scaffolds integrated well with the host tissue with no obvious gap between the implants and the injured sites. In addition, large numbers of host cells migrated into the scaffolds and extensive blood vessel formation was observed in the implants. Transplanted Schwann cells and NPCs survived after 6 weeks post-implantation, with some Schwann cells maturing with tube-like morphologies and some NPCs differentiating into neurons, astrocytes and oligodendrocytes. Immunolabeling further revealed more axonal growth into the implants with SCs or NPCs than in the case of plain sapeptide scaffolds.

For CNS repair, astroglial formation is the major obstacle to axonal regeneration after spinal cord injuries, thus inhibition of glial scar formation is crucial for successful axonal regeneration [4,89,90]. Tysseling-Mattiace et al. demonstrated the possibility of inhibiting glial scar formation and promoting axon elongation after spinal cord injury by using IKVAV sapeptide scaffolds. The spinal cords of female mice were compressed dorsoventrally by the extradural application of a 24 g modified aneurysm clip for 1 min. Following that, sapeptide solution was injected 24 h after injury. At 5 weeks and 11 weeks post-treatment, a significant reduction of astrogliosis was found in the animals that received IKVAVA sapeptide nanofibers. In contrast, no reduction was found in animals that were injected with a peptide, glutamic acid–glutamine–serine (EQS), which is non-bioactive and does not support neural cell differentiation and neurite outgrowth [57]. Ten days after injury, a decreased incidence of oligodendroglial (OL) death was found in the area adjacent to the lesion site after IKVAV sapeptide treatment. Functional recovery as assessed 9 weeks after injury using the Basso, Beattie and Bresnahan (BBB) locomotor scale modified for the mouse [91], indicated enhanced recovery in the IKVAV sapeptide group as compared to control groups of EQS sapeptide, sham injection, and glucose delivery.

The feasibility of using SAPNSs for brain injury repair was demonstrated by Ellis-Behnke et al. [92]. After creating a knife wound of 1.5 mm deep and 2.0 mm wide in the midbrain in P2 hamsters, SAPNS solutions were applied within the wounded area. Thirty days after injury, dense projections were observed to cross the SAPNS-treated lesion sites, whilst empty cavities with no axonal regeneration were formed in animals that were left untreated post-injury. The efficacy of SAPNSs in enhancing CNS regeneration in optical tract injuries was also evaluated by the same group. Following the transection of the optic tract at the brachium of the superior colliculus (SC), SAPNS solutions were injected into the injured sites. Histological results showed that tissue reconnection across the injured sites of SAPNSs-treated animals occurred at all time points of 30, 45 and 90 days. Behavioral tests for evaluating functional vision recovery performed after 90 days treatment showed that 75% of visual ability had returned in the experimental group, whilst animals in the control group that were treated with saline remained blind. These results supported the possibility of using designed sapeptide scaffolds to promote axonal regeneration with functional recovery in the CNS.

Although many *in vitro* and *in vivo* experiments have proved the advantages of using sapeptide nanofibrous scaffolds in neural tissue engineering, the technique of self-assembly has its own limitation of forming uncontrollably macro-sized pores and mechanically-unstable 3D structures [93]. Such are perhaps reasons for the common application of SAPNSs to CNS injury treatment and the lack of its use as a PNS nerve regeneration approach as more stringent requirements over scaffold mechanical properties often exist for PNS regeneration. Compared with electrospinning, more complicated procedures and techniques are often required by self-assembly, which may potentially lead to lower productivity [94]. However, despite these drawbacks, self-assembled nanofiber still retains its own attractiveness due to its true nano-sized biomimicking architectural features that have thus far been unattainable using electrospinning.

## 4. Conclusion

Nerve regeneration is a complex process and a challenging field for researchers. From our limited knowledge of the nervous system to the achievement of partial neural functional recovery, impressive progress has been made during the past decades. However, much

remains unexplored and the journey to accomplishing the final goal of fully regenerating functional nervous systems continues.

While topographical features from sub-micron- and nanofibers, aligned or randomly-oriented, clearly affect neural cell morphology and function, detailed in-depth studies are still required. In particular, our current understanding of the effects of fibrous architecture on cellular function is still limited to the evaluation of cell morphological and viability changes. Detailed mechanistic evaluation of cellular functions such as Schwann cell myelination, glial scar formation, neuron synapse formation, neural cell signal transduction and neural stem cell differentiation is required to facilitate the precise control of cellular functions by nanofibrous topographical signals. The size effect of topographical features on cellular function is also unknown. The ideal size of nanofibers that should be coupled with the cell type of interest remains to be elucidated and such may only be found when other parameters such as material chemistry are decoupled from the experimentations. Finally, in terms of *in vivo* studies, minimization of the variability in test methods used by different groups will also enable more fruitful comparisons between experimental outcomes.

Nanofibrous scaffolds can serve as powerful tools in the tissue regeneration processes. The fibrous structure closely resembles the natural environment that cells grow in and provides appropriate physical cues for manipulating cellular functions. Electrospinning is a versatile and economical way of mass-producing fibrous constructs. It also enables incorporation of biochemicals to provide a synergistic effect in promoting nerve regeneration. Self-assembled nanofibers, though when using current available techniques are synthesized less efficiently, can provide a true biomimicking platform with their nanofibrous architecture. Altogether, such biofunctional nanofibrous scaffolds hold great potential as direct implantable devices and as basic neural cell biological study platforms that may be necessary for neural tissue engineering.

## Acknowledgments

The authors would like to thank the partial funding support of this work by A*STAR BMRC (07/1/22/19/519) and NMRC (EDG07nov016).

## References

[1] T.E. Trumble, F.G. Shon, The physiology of nerve transplantation, Hand Clin. 16 (2000) 105–122.
[2] P.J. Evans, R. Midha, S.E. Mackinnon, The peripheral nerve allograft: a comprehensive review of regeneration and neuroimmunology, Prog. Neurobiol. 43 (1994) 187–233.
[3] J.L. Platt, G.M. Vercellotti, A.P. Dalmasso, A.J. Matas, R.M. Bolman, J.S. Najarian, F.H. Bach, Transplantation of discordant xenografts: a review of progress, Immunol. Today 11 (1990) 450–456.
[4] J.W. Fawcett, R.A. Asher, The glial scar and central nervous system repair, Brain Res. Bull. 49 (1999) 377–391.
[5] A. Jain, Y.T. Kim, R.J. McKeon, R.V. Bellamkonda, In situ gelling hydrogels for conformal repair of spinal cord defects, and local delivery of BDNF after spinal cord injury, Biomaterials. 27 (2006) 497–504.
[6] J. Taipale, J. Keski-Oja, Growth factors in the extracellular matrix, FASEB J. 11 (1997) 51–59.
[7] F. Berthiaume, P.V. Moghe, M. Toner, M.L. Yarmush, Effect of extracellular matrix topology on cell structure, function, and physiological responsiveness: hepatocytes cultured in a sandwich configuration, FASEB J. 10 (1996) 1471–1484.
[8] D. Liang, B.S. Hsiao, B. Chu, Functional electrospun nanofibrous scaffolds for biomedical applications, Adv. Drug Deliv. Rev. 59 (2007) 1392–1412.
[9] C. Li, C. Vepari, H.J. Jin, H.J. Kim, D.L. Kaplan, Electrospun silk-BMP-2 scaffolds for bone tissue engineering, Biomaterials. 27 (2006) 3115–3124.
[10] E.R. Kenawy, G.L. Bowlin, K. Mansfield, J. Layman, D.G. Simpson, E.H. Sanders, G.E. Wnek, Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic acid), and a blend, J. Control. Release. 81 (2002) 57–64.
[11] Y.K. Luu, K. Kim, B.S. Hsiao, B. Chu, M. Hadjiargyrou, Development of a nanostructured DNA delivery scaffold via electrospinning of PLGA and PLA-PEG block copolymers, J. Control. Release. 89 (2003) 341–353.
[12] S.Y. Chew, Y. Wen, Y. Dzenis, K.W. Leong, The role of electrospinning in the emerging field of nanomedicine, Curr. Pharm. Des. 12 (2006) 4751–4770.
[13] R. Murugan, S. Ramakrishna, Nano-featured scaffolds for tissue engineering: a review of spinning methodologies, Tissue Eng. 12 (2006) 435–447.
[14] J. Venugopal, S. Low, A.T. Choon, S. Ramakrishna, Interaction of cells and nanofiber scaffolds in tissue engineering, J. Biomed. Mater. Res. B. 84 (2008) 34–48.
[15] W.H.S.R. Wee-Eong Teo, Electrospun scaffold tailored for tissue-specific extracellular matrix, Biotechno. J. 1 (2006) 918–929.
[16] C.P. Barnes, S.A. Sell, E.D. Boland, D.G. Simpson, G.L. Bowlin, Nanofiber technology: designing the next generation of tissue engineering scaffolds, Adv. Drug Deliv. Rev. 59 (2007) 1413–1433.
[17] R. Murugan, S. Ramakrishna, Design strategies of tissue engineering scaffolds with controlled fiber orientation, Tissue Eng. 13 (2007) 1845–1866.
[18] S. Heydarkhan-Hagvall, K. Schenke-Layland, A.P. Dhanasopon, F. Rofail, H. Smith, B.M. Wu, R. Shemin, R.E. Beygui, W.R. MacLellan, Three-dimensional electrospun ECM-based hybrid scaffolds for cardiovascular tissue engineering, Biomaterials. 29 (2008) 2907–2914.
[19] W.J. Li, R.L. Mauck, J.A. Cooper, X. Yuan, R.S. Tuan, Engineering controllable anisotropy in electrospun biodegradable nanofibrous scaffolds for musculoskeletal tissue engineering, J. Biomech. 40 (2007) 1686–1693.
[20] W.J. Li, R. Tuli, X. Huang, P. Laquerriere, R.S. Tuan, Multilineage differentiation of human mesenchymal stem cells in a three-dimensional nanofibrous scaffold, Biomaterials. 26 (2005) 5158–5166.
[21] F. Yang, C.Y. Xu, M. Kotaki, S. Wang, S. Ramakrishna, Characterization of neural stem cells on electrospun poly(L-lactic acid) nanofibrous scaffold, J. Biomater. Sci-Polym E. 15 (2004) 1483–1497.
[22] D.R. Nisbet, S. Pattanawong, N.E. Ritchie, N.E. Ritchie, N.E. Ritchie, N.E. Ritchie, W. Shen, D.I. Finkelstein, M.K. Horne, J.S. Forsythe, Interaction of embryonic cortical neurons on nanofibrous scaffolds for neural tissue engineering, J. Nerual Eng. 4 (2007) 35.
[23] O. Suwantong, S. Waleetorncheepsawat, N. Sanchavanakit, P. Pavasant, P. Cheepsunthorn, T. Bunaprasert, P. Supaphol, In vitro biocompatibility of electrospun poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) fiber mats, Int. J. Biol. Macromol. 40 (2007) 217–223.
[24] S. Meiners, I. Ahmed, A.S. Ponery, N. Amor, S.L. Harris, V. Ayres, Y. Fan, Q. Chen, R. Delgado-Rivera, A.N. Babu, Engineering electrospun nanofibrillar surfaces for spinal cord repair: a discussion, Polym. Int. 56 (2007) 1340–1348.
[25] S. Panseri, C. Cunha, J. Lowery, U. Del Carro, F. Taraballi, S. Amadio, A. Vescovi, F. Gelain, Electrospun micro- and nanofiber tubes for functional nervous regeneration in sciatic nerve transections, BMC Biotech. 8 (2008) 39.
[26] J.M. Corey, C.C. Gertz, B.-S. Wang, L.K. Birrell, S.L. Johnson, D.C. Martin, E.L. Feldman, The design of electrospun PLLA nanofiber scaffolds compatible with serum-free growth of primary motor and sensory neurons, Acta Biomater. 4 (2008) 863–875.
[27] W. Wang, S. Itoh, A. Matsuda, S. Ichinose, K. Shinomiya, Y. Hata, J. Tanaka, Influences of mechanical properties and permeability on chitosan nano/microfiber mesh tubes as a scaffold for nerve regeneration, J. Biomed. Mater. Res. A. 84A (2008) 557–566.
[28] P.A. Gunatillake, R. Adhikari, N. Gadegaard, Biodegradable synthetic polymers for tissue engineering, Eur Cells Mater. 5 (2003) 1–16.
[29] P. Sangsanoh, S. Waleetorncheepsawat, O. Suwantong, P. Wutticharoenmongkol, O. Weeranantanapan, B. Chuenjitbuntaworn, P. Cheepsunthorn, P. Pavasant, P. Supaphol, In vitro biocompatibility of Schwann cells on surfaces of biocompatible polymeric electrospun fibrous and solution-cast film scaffolds, Biomacromolecules 8 (2007) 1587–1594.
[30] S.M. Willerth, S.E. Sakiyama-Elbert, Approaches to neural tissue engineering using scaffolds for drug delivery, Adv. Drug Deliv. Rev. 59 (2007) 325–338.
[31] J.A. Matthews, G.E. Wnek, D.G. Simpson, G.L. Bowlin, Electrospinning of collagen nanofibers, Biomacromolecules 3 (2002) 232–238.
[32] Z.M. Huang, Y.Z. Zhang, S. Ramakrishna, C.T. Lim, Electrospinning and mechanical characterization of gelatin nanofibers, Polymer. 45 (2004) 5361–5368.
[33] K. Ohkawa, D. Cha, H. Kim, A. Nishida, H. Yamamoto, Electrospinning of chitosan, Macromol. Rapid. Commun. 25 (2004) 1600–1605.
[34] W. Wang, S. Itoh, A. Matsuda, T. Aizawa, M. Demura, S. Ichinose, K. Shinomiya, J. Tanaka, Enhanced nerve regeneration through a bilayered chitosan tube: the effect of introduction of glycine spacer into the CYIGSR sequence, J. Biomed. Mater. Res. A. 85A (2008) 919–928.
[35] I. Ahmed, H.-Y. Liu, P.C. Mamiya, A.S. Ponery, A.N. Babu, T. Weik, M. Schindler, S. Meiners, Three-dimensional nanofibrillar surfaces covalently modified with tenascin-C-derived peptides enhance neuronal growth in vitro, J. Biomed. Mater. Res. A. 76A (2006) 851–860.
[36] S. Meiners, M.S.A. Nur-E-Kamal, M.L.T. Mercado, Identification of a neurite outgrowth-promoting motif within the alternatively spliced region of human tenascin-C, J. Neurosci. 21 (2001) 7215–7225.
[37] L. Wensheng, Guo Ying, Wang Hui, Shi Dejin, Liang Chaofeng, Ye Zhuopeng, Qing Feng, Gong Jin, Electrospun nanofibers immobilized with collagen for neural stem cells culture, J. Mater. Sci-Mater M 19 (2008) 847–854.
[38] S. Patel, K. Kurpinski, R. Quigley, H. Gao, B.S. Hsiao, M.M. Poo, S. Li, Bioactive nanofibers: synergistic effects of nanotopography and chemical signaling on cell guidance, Nano Lett. 7 (2007) 2122–2128.
[39] M.P. Prabhakaran, J.R. Venugopal, T.T. Chyan, L.B. Hai, C.K. Chan, A.Y. Lim, S. Ramakrishna, Electrospun biocomposite nanofibrous scaffolds for neural tissue engineering, Tissue Eng. — Part A. 14 (2008) 1787–1797.
[40] P.B. Van Wachem, T. Beugeling, J. Feijen, Interaction of cultured human endothelial cells with polymeric surfaces of different wettabilities, Biomaterials 6 (1985) 403–408.
[41] L. Ghasemi-Mobarakeh, M. P. Prabhakaran, M. Morshed, M.-H. Nasr-Esfahani, S. Ramakrishna, Electrospun poly(ε-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering, Biomaterials. In Press, Corrected Proof.
[42] E. Schnell, K. Klinkhammer, S. Balzer, G. Brook, D. Klee, P. Dalton, J. Mey, Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly-[ε]-

caprolactone and a collagen/poly-[ε]-caprolactone blend, Biomaterials 28 (2007) 3012–3025.
[43] H.S. Koh, T. Yong, C.K. Chan, S. Ramakrishna, Enhancement of neurite outgrowth using nano-structured scaffolds coupled with laminin, Biomaterials 29 (2008) 3574–3582.
[44] C. Miller, H. Shanks, A. Witt, G. Rutkowski, S. Mallapragada, Oriented Schwann cell growth on micropatterned biodegradable polymer substrates, Biomaterials 22 (2001) 1263–1269.
[45] F. Johansson, P. Carlberg, N. Danielsen, L. Montelius, M. Kanje, Axonal outgrowth on nano-imprinted patterns, Biomaterials 27 (2006) 1251–1258.
[46] J.M. Corey, D.Y. Lin, K.B. Mycek, Q. Chen, S. Samuel, E.L. Feldman, D.C. Martin, Aligned electrospun nanofibers specify the direction of dorsal root ganglia neurite growth, J. Biomed. Mater. Res. A. 83A (2007) 636–645.
[47] W.N. Chow, D.G. Simpson, J.W. Bigbee, R.J. Colello, Evaluating neuronal and glial growth on electrospun polarized matrices: bridging the gap in percussive spinal cord injuries, Neuron Glia Biol. 3 (2007) 119–126.
[48] S.Y. Chew, R. Mi, A. Hoke, K.W. Leong, The effect of the alignment of electrospun fibrous scaffolds on Schwann cell maturation, Biomaterials 29 (2008) 653–661.
[49] M. Lietz, L. Dreesmann, M. Hoss, S. Oberhoffner, B. Schlosshauer, Neuro tissue engineering of glial nerve guides and the impact of different cell types, Biomaterials 27 (2006) 1425–1436.
[50] D.E. Ingber, J. Folkman, Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis in vitro: role of extracellular matrix, J. Cell Biol. 109 (1989) 317–330.
[51] H.J. Kong, J. Liu, K. Riddle, T. Matsumoto, K. Leach, D.J. Mooney, Non-viral gene delivery regulated by stiffness of cell adhesion substrates, Nat Mater. 4 (2005) 460–464.
[52] D.E. Ingber, Mechanical control of tissue growth: function follows form, Proc. Nat. Acad. Sci. U.S.A. 102 (2005) 11571–11572.
[53] L.L. Jones, M. Oudega, M.B. Bunge, M.H. Tuszynski, Neurotrophic factors, cellular bridges and gene therapy for spinal cord injury, J. Physiol. (Lond) 533 (2001) 83–89.
[54] F.H. Gage, Mammalian neural stem cells, Science 287 (2000) 1433–1438.
[55] F. Yang, R. Murugan, S. Wang, S. Ramakrishna, Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering, Biomaterials 26 (2005) 2603–2610.
[56] J. Xie, S.M. Willerth, X. Li, M.R. Macewan, A. Rader, S.E. Sakiyama-Elbert, Y. Xia, The differentiation of embryonic stem cells seeded on electrospun nanofibers into neural lineages, Biomaterials 30 (2009) 354–362.
[57] G.A. Silva, C. Czeisler, K.L. Niece, E. Beniash, D.A. Harrington, J.A. Kessler, S.I. Stupp, Selective differentiation of neural progenitor cells by high-epitope density nanofibers, Science 303 (2004) 1352–1355.
[58] A. Markus, T.D. Patel, W.D. Snider, Neurotrophic factors and axonal growth, Curr. Opin. Neurobiol. 12 (2002) 523–531.
[59] J. Boyd, T. Gordon, Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury, Mol. Neurobiol. 27 (2003) 277–323.
[60] C. Xudong, M.S. Shoichet, Delivering neuroactive molecules from biodegradable microspheres for application in central nervous system disorders, Biomaterials 20 (1999) 329–339.
[61] Y.C. Wang, Y.T. Wu, H.Y. Huang, H.I. Lin, L.W. Lo, S.F. Tzeng, C.S. Yang, Sustained intraspinal delivery of neurotrophic factor encapsulated in biodegradable nanoparticles following contusive spinal cord injury, Biomaterials 29 (2008) 4546–4553.
[62] E.G. Fine, I. Decosterd, M. Papaloïzos, A.D. Zurn, P. Aebischer, GDNF and NGF released by synthetic guidance channels support sciatic nerve regeneration across a long gap, Eur. J. NeuroSci. 15 (2002) 589–601.
[63] J. Bloch, E.G. Fine, N. Bouche, A.D. Zurn, P. Aebischer, Nerve growth factor- and neurotrophin-3-releasing guidance channels promote regeneration of the transected rat dorsal root, Exp. Neurol. 172 (2001) 425–432.
[64] S.Y. Chew, J. Wen, E.K.F. Yim, K.W. Leong, Sustained release of proteins from electrospun biodegradable fibers, Biomacromolecules 6 (2005) 2017–2024.
[65] S.Y. Chew, R. Mi, A. Hoke, K.W. Leong, Aligned protein–polymer composite fibers enhance nerve regeneration: a potential tissue-engineering platform, Adv. Funct. Mater. 17 (2007) 1288–1296.
[66] S. Kidoaki, I.K. Kwon, T. Matsuda, Mesoscopic spatial designs of nano- and microfiber meshes for tissue-engineering matrix and scaffold based on newly devised multilayering and mixing electrospinning techniques, Biomaterials 26 (2005) 37–46.
[67] Y.-T. Kim, V.K. Haftel, S. Kumar, R.V. Bellamkonda, The role of aligned polymer fiber-based constructs in the bridging of long peripheral nerve gaps, Biomaterials 29 (2008) 3117–3127.
[68] G.M. Whitesides, B. Grzybowski, Self-assembly at all scales, Science 295 (2002) 2418–2421.
[69] S. Zhang, Fabrication of novel biomaterials through molecular self-assembly, Nat. Biotechol. 21 (2003) 1171–1178.
[70] M. Fandrich, M.A. Fletcher, C.M. Dobson, Amyloid fibrils from muscle myoglobin, Nature 410 (2001) 165–166.
[71] D.G. Lynn, S.C. Meredith, Review: model peptides and the physicochemical approach to [beta]-amyloids, J. Struct. Biol. 130 (2000) 153–173.
[72] M.D. Shtilerman, T.T. Ding, P.T. Lansbury, Molecular crowding accelerates fibrillization of α-synuclein: could an increase in the cytoplasmic protein concentration induce Parkinson's disease, Biochemistry 41 (2002) 3855–3860.
[73] M. Reches, Y. Porat, E. Gazit, Amyloid fibril formation by pentapeptide and tetrapeptide fragments of human calcitonin, J. Biol. Chem. 277 (2002) 35475–35480.
[74] K.H.J. Buschow, Encyclopedia of Materials: Science and Technology, Elesevier, New York, 2001.
[75] F. Gelain, D. Bottai, A. Vescovi, S. Zhang, Designer self-assembling peptide nanofiber scaffolds for adult mouse neural stem cell 3-dimensional cultures, PLoS ONE 1 (2006).
[76] G. Rosenblat, N. Perelman, E. Katzir, S. Gal-Or, A. Jonas, M.E. Nimni, N. Sorgente, I. Neeman, Acylated ascorbate stimulates collagen synthesis in cultured human foreskin fibroblasts at lower doses than does ascorbic acid, Connect. Tissue Res. 37 (1998) 303–311.
[77] A. Firth, A. Aggeli, J.L. Burke, X. Yang, J. Kirkham, Biomimetic self-assembling peptides as injectable scaffolds for hard tissue engineering, Nanomedicine (London, England) 1 (2006) 189–199.
[78] E. Kokkoli, A. Mardilovich, A. Wedekind, E.L. Rexeisen, A. Garg, J.A. Craig, Self-assembly and applications of biomimetic and bioactive peptide-amphiphiles, Soft Matter. 2 (2006) 1015–1024.
[79] P.A. Madurantakam, C.P. Cost, D.G. Simpson, G.L. Bowlin, Science of nanofibrous scaffold fabrication: strategies for next generation tissue-engineering scaffolds, Nanomedicine 4 (2009) 193–206.
[80] T.C. Holmes, S. De Lacalle, X. Su, G. Liu, A. Rich, S. Zhang, Extensive neurite outgrowth and active synapse formation on self-assembling peptide scaffolds, Proc. Nat. Acad. Sci. U.S.A. 97 (2000) 6728–6733.
[81] C.E. Semino, J. Kasahara, Y. Hayashi, S. Zhang, Entrapment of migrating hippocampal neural cells in three-dimensional peptide nanofiber scaffold, Tissue Eng. 10 (2004) 643–655.
[82] H. Hosseinkhani, M. Hosseinkhani, H. Kobayashi, Proliferation and differentiation of mesenchymal stem cells using self-assembled peptide amphiphile nanofibers, Biomed. Mater. 1 (2006) 8–15.
[83] L. Kam, W. Shain, J.N. Turner, R. Bizios, Axonal outgrowth of hippocampal neurons on micro-scale networks of polylysine-conjugated laminin, Biomaterials 22 (2001) 1049–1054.
[84] S.K. Powell, J. Rao, E. Roque, M. Nomizu, Y. Kuratomi, Y. Yamada, H.K. Kleinman, Neural cell response to multiple novel sites on laminin-1, J. Neurosci. Res. 61 (2000) 302–312.
[85] M. Parmar, C. Skogh, A. Björklund, K. Campbell, Regional specification of neurosphere cultures derived from subregions of the embryonic telencephalon, Mol. Cell. Neurosci. 21 (2002) 645–656.
[86] S. Wu, Y. Suzuki, Y. Ejiri, T. Noda, H. Bai, M. Kitada, K. Kataoka, M. Ohta, H. Chou, C. Ide, Bone marrow stromal cells enhance differentiation of cocultured neurosphere cells and promote regeneration of injured spinal cord, J. Neurosci. Res. 72 (2003) 343–351.
[87] E. Alsberg, K.W. Anderson, A. Albeiruti, J.A. Rowley, D.J. Mooney, Engineering growing tissues, Proc. Nat. Acad. Sci. U.S.A. 99 (2002) 12025–12030.
[88] J. Guo, H. Su, Y. Zeng, Y.X. Liang, W.M. Wong, R.G. Ellis-Behnke, K.F. So, W. Wu, Reknitting the injured spinal cord by self-assembling peptide nanofiber scaffold, Nanomedicine: Nanotechnology, Biol. Med. 3 (2007) 311–321.
[89] M. Shearer, J. Fawcett, The astrocyte/meningeal cell interface — a barrier to successful nerve regeneration? Cell Tissue Res. 305 (2001) 267–273.
[90] E.J. Bradbury, L.D.F. Moon, R.J. Popat, V.R. King, G.S. Bennett, P.N. Patel, J.W. Fawcett, S.B. McMahon, Chondroitinase ABC promotes functional recovery after spinal cord injury, Nature 416 (2002) 636–640.
[91] D.M. Basso, et al., MASCIS evaluation of open field locomoter scores: effects of experience and teamwork on reliability, J. Neurotraum. 13 (1996) 343–359.
[92] R.G. Ellis-Behnke, Y.X. Liang, S.W. You, D.K.C. Tay, S. Zhang, K.F. So, G.E. Schneider, Nano neuro knitting: peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision, Proc. Nat. Acad. Sci. U.S.A. 103 (2006) 5054–5059.
[93] H. Shin, S. Jo, A.G. Mikos, Biomimetic materials for tissue engineering, Biomaterials 24 (2003) 4353–4364.
[94] Z. Ma, M. Kotaki, R. Inai, S. Ramakrishna, Potential of nanofiber matrix as tissue-engineering scaffolds, Tissue Eng. 11 (2005) 101–109.
[95] I.A. Kim, S.A. Park, Y.J. Kim, S.-H. Kim, H.J. Shin, Y.J. Lee, S.G. Kang, J.-W. Shin, Effects of mechanical stimuli and microfiber-based substrate on neurite outgrowth and guidance, Soc. Biotechnol. Japan. 101 (2006) 120–126.
[96] V.M. Tysseling-Mattiace, V. Sahni, K.L. Niece, D. Birch, C. Czeisler, M.G. Fehlings, S.I. Stupp, J.A. Kessler, Self-assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury, J. Neurosci. 28 (2008) 3814–3823.