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To cite this article: Anuradha Subramanian *et al* 2011 *Biomed. Mater.* **6** 025004

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Fabrication of uniaxially aligned 3D electrospun scaffolds for neural regeneration

Anuradha Subramanian^{1,2}, Uma Maheswari Krishnan^{1,2}
and Swaminathan Sethuraman^{1,2,3}

¹ Center for Nanotechnology & Advanced Biomaterials, SASTRA University, Thanjavur 613 401, Tamil Nadu, India

² School of Chemical & Biotechnology, SASTRA University, Thanjavur 613 401, Tamil Nadu, India

E-mail: swami@sastra.edu

Received 28 October 2010

Accepted for publication 18 January 2011

Published 7 February 2011

Online at stacks.iop.org/BMM/6/025004

Abstract

Nanofibrous scaffolds are very promising physical guidance substrates for regenerating nerves to traverse larger nerve gaps. In this study, we have attempted to develop 2D random and 3D longitudinally oriented nanofibers of poly(lactide-co-glycolide) (PLGA) by the modified electrospinning process and characterized the surface morphology, mechanical properties, porosity, degradation and wettability. The orientation of aligned fibers was optimized by varying the speed of the rotating mandrel in the electrospinning process. The mean diameter of random PLGA nanofibers was 197 ± 72 nm, whereas that of the aligned PLGA fiber was 187 ± 121 nm. The pore size of aligned PLGA nanofibers (3.5 ± 1.1 μm) was significantly lower than their respective random nanofibers (8.0 ± 2.0 μm) ($p < 0.05$). However, the percentage porosity of both scaffolds was comparable ($p > 0.05$). The mass loss percentage and molecular weight loss percentage due to degradation was higher in random PLGA fibers when compared to aligned PLGA after 5 weeks ($p < 0.05$). The tensile strength and Young's modulus of random PLGA fibers were significantly higher than those of the aligned PLGA nanofibers ($p < 0.05$). Both random and longitudinally aligned scaffolds were used for the *in vitro* culture of Schwann cells. Morphology and cell proliferation results demonstrated that the aligned fibers assist the direction of Schwann cells and a better proliferation rate than their random fibers. The results confirmed that aligned nanofibers have better deformability, slow degradation, comparable porosity and orientation cues than random nanofibers. Hence the longitudinally aligned nanofibers may be ideal scaffolds for nerve regeneration.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Nerve injury is a serious clinical complication which requires immediate intervention to restore the functions since the matured neurons do not undergo cell division. In a small nerve defect the axons can bridge the gap through the formation of fibrin cable and Bungner bands [1]. However, in larger lesions, denuded peripheral nervous system axons

are unable to regenerate inherently [2]. The current gold standard approach of using autografts to bridge larger gaps has limitations such as painful neuroma formation, functional loss at the donor site, compliance mismatch, etc [3]. Hence, development of artificial nerve grafts offers a viable alternative to the conventional autografts to treat neural defects [4].

After peripheral nerve injury, glial cells migrate toward the distal end of smaller gaps to direct the neurite outgrowth [5]. In larger nerve gaps, the fibrin cable and Bungner band formation are compromised [6]. Therefore, it is desirable

³ Author to whom any correspondence should be addressed.

to treat the larger nerve gaps using a scaffold having well-defined architecture to orient the glial cells longitudinally toward the distal end while acting as physical template to bridge the gap. Thus the proposed scaffold should be tubular with desired topographical cue (uniaxial alignment fibers), compliant match to the native nerves in order to orient the glial cells toward the distal end [7].

Electrospinning is a well-known technique for the fabrication of nanofibrous scaffolds for tissue engineering applications [8]. Electrospun nanofibrous scaffolds have received much attention in tissue engineering due to the resemblance with collagen fibers in extracellular matrix, high surface area to volume ratio, high porosity, and tailorable mechanical and degradable properties [7, 9–11]. Recent studies have demonstrated that the direction of the aligned nanofiber scaffold determines the path for neurite extension [12]. Poly(lactide-co-glycolide) (PLGA) random nanofibers and microfibers, aligned microfibers and films were investigated for the C17.2 neural stem cell culture and the differentiation of neurons along the fiber direction toward the treatment of neural defects [13] was recognized. Aligned electrospun collagen/(poly(ϵ -caprolactone)(PCL) fibers support cell proliferation, glial migration, and orientation of neurite outgrowth, suggesting their suitability as nerve implants [7]. Aligned electrospun PCL fibers have been found to up-regulate specific genes such as P0 and down-regulate NCAM-1 in cultured Schwann cells thereby promoting the Schwann cell maturation [12]. Significantly higher Schwann cell migration and neurite outgrowth were observed in uniaxially aligned fibers of poly(acrylonitrile-co-methylacrylate) (PAN-MA) developed by electrospinning than in random fibers [14].

Recently a tubular scaffold has been proved as a promising substrate for use in tissue engineering due to its uniform thickness, properties and mechanical strength all over the tube [15]. However, the orientation of electrospun nanofibers is a challenge due to their intricate three-dimensional whipping motion of a jet [16]. A high-speed rotating drum has been used to align the fibers circumferentially [17]. However, the complaint mismatch of the graft and partial alignment of fibers limit their use in neural regeneration [18]. A thin disk with a sharp edge collector has been found to improve the orientation of fibers [19]. However, fibers were collected only at the edge of the disk. The use of auxiliary electrodes as a collector has a profound effect on the orientation of fibers [18]. A charged jet experiences a strong stretching force between the electrodes when it reaches the collector. This facilitates the formation of suspended arrays of aligned fibers across the gap. However, developing a thicker layer and longer length of aligned fibers is very difficult [18].

Among the synthetic biodegradable polymers, PLGA has been chosen for this study as it is most extensively investigated for neural regeneration and also because of its established biocompatible and biodegradable properties [13]. In addition to this, PLGA has shown better performance in terms of cell viability, apoptotic activity, mitochondrial metabolic activity, proliferation and differentiation rate of HiB5 cells than poly(L-lactic acid) (PLLA) and (poly(lactide-co- ϵ -caprolactone) (PLCL) [20]. In this study, we have

proposed a simple electrospinning setup to obtain a tubular structure with longitudinally aligned nanofibers. Using such setup, we have developed a longitudinally aligned PLGA nanofibrous scaffold. The properties of a 3D aligned scaffold such as porosity, mechanical strength, wettability and biodegradability were investigated and compared with respect to a 2D random mat to explore its applications on axonal regeneration. The rat Schwann cell adhesion and proliferation on both 2D and 3D scaffolds were investigated to investigate 3D scaffold applications in neural tissue engineering.

2. Materials and methods

2.1. Materials

PLGA (M_w -118 kDa) was purchased from Lakeshore Biomaterials, Birmingham, USA. Dichloromethane (DCM) and N,N-dimethyl formamide (DMF) were purchased from Merck, India and used without any further purification.

2.2. Fabrication of random and aligned nanofibrous scaffolds

The 2D random and 3D aligned PLGA nanofibers were developed through electrospinning under optimum conditions. Briefly, 12% (w/v) of PLGA was dissolved in a mixture of DCM and DMF (9:1). This solution was loaded into a 5 mL glass syringe and the flow rate of $0.001 \text{ mL min}^{-1}$ was controlled by syringe pump (KD Scientific 200, USA). A high voltage (25 kV) was applied using a high-voltage power supply (Zeonics, India). The 2D random fibers were collected on aluminum foil fixed onto a grounded metal collector.

The 3D axially aligned nanofibers were collected using a grounded rotating mandrel (3 mm in diameter) with small insulating gaps (figure 1). Briefly, a small insulating sleeve (1.5 cm in length) was introduced into the rotating metal rod (4 mm in diameter) to create an alternating metal and insulating part, and the metal rod was connected with ground. The insulating gap between the grounded metal rod behaves as an auxiliary electrode which allows the deposition of longitudinally oriented fibers onto the insulating portion of the rod. The speed of the mandrel was varied from 1000 to 2500 rpm using a motor (Remi, India) to improve the fiber orientation, while other parameters such as concentration, flow rate, applied voltage and tip–target distance were maintained at 12% (w/v), $0.001 \text{ mL min}^{-1}$, 25 kV and 12 cm respectively. These electrospun nanofibers were stored in a vacuum desiccator for further characterization.

2.3. Surface morphology

The surface morphology, diameter of the fibers and orientation of aligned fibers were determined using a scanning electron microscope (SEM, JEOL FE-SEM 6701F, Japan). The electrospun nanofibers were sputter coated with platinum and examined under SEM at an accelerating voltage of 3 kV. The distribution of fiber orientation was analyzed by calculating the relative angle of individual fibers from $+90^\circ$ to -90° where 0° was taken as the axial direction.

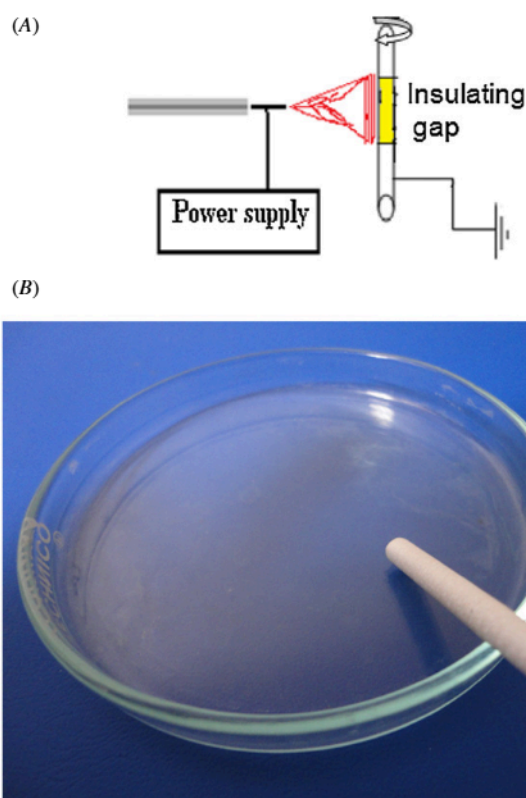


Figure 1. (A) Electrospinning setup used for the fabrication of the longitudinally oriented tubular scaffold. (B) Tubular scaffold.

2.4. Contact angle measurement

The water contact angle of both 2D random and 3D aligned nanofibers was measured using a goniometer (Rame-Hart, USA). Approximately 5 μL of deionized water was carefully placed on to the surface of electrospun fiber and contact angle values were calculated ($n = 5$).

2.5. Mechanical testing

Mechanical properties of 2D random and longitudinally aligned 3D PLGA scaffolds were determined using an Instron uniaxial tensile testing machine (INSTRON, USA). The 2D specimens were cut into dumbbell shape with a width of 10 mm, gauge length of 50 mm and thickness ranging from 0.17 to 0.2 mm ($n = 6$). Three-dimensional samples of 50 mm length and 5 mm outer diameter were taken for measurement ($n = 6$). Load deformation data were recorded at a deforming speed of 1 mm s^{-1} , and the stress–strain curve of nanofibers was constructed from the load deformation curve.

2.6. Porosity measurement

The porosity and distribution of pore size of both 2D random mats and 3D aligned scaffolds were measured using mercury intrusion porosimeter (Micromeritics Autopore IV, USA). The 2D random PLGA scaffold (0.2 mm thickness) was cut into 2 cm $\times$ 5 cm rectangular shapes and weighed ($n = 3$). For the 3D aligned scaffold, samples of 3 mm inner diameter and 5 cm length were taken for analysis ($n = 3$). The pressure was

varied from 0.1 to 50 psi with the equilibration time of 60 s for each intermediate data point. The pore size and porosity of all scaffolds were determined from the amount of mercury that penetrated into the sample at different pressures.

2.7. In vitro degradation study

The 2D random electrospun membranes were cut into a square shape with dimensions of 40 mm $\times$ 40 mm for *in vitro* degradation ($n = 3$). The 3D aligned fibers with 3 mm inner diameter and 20 mm length were used for degradation study ($n = 3$). The weight of the 2D random and 3D aligned fibers was measured and the fibers were immersed in 30 mL phosphate buffered saline (PBS) at pH 7.4 and 37 $^{\circ}\text{C}$ with constant shaking using a water bath shaker (LABLINE, India), and the PBS solution was changed every 3 days. At predetermined time points (1, 2, 3, 4 and 5 weeks) the samples were removed and rinsed with deionized water, dabbed with tissue paper to remove surface water and dried under vacuum for 1 week. The dry weights of both the 2D random and 3D aligned nanofibers were recorded and the surface morphology of degraded sample was characterized using a SEM. The weight loss percentage of each sample was calculated by the following formula:

$$\text{Weight loss percentage} = \frac{\text{initial weight of sample} - \text{dry weight of degraded sample}}{\text{initial weight of sample}} \times 100.$$

The molecular weight loss of both polymeric scaffolds at different time points was characterized using gel permeation chromatography (GPC, Agilent 1200 Series, USA) by injecting 50 μL of each sample. The samples were dissolved in THF to make a 1% solution and eluted at a flow rate of 1.5 mL min^{-1} through a size exclusion column calibrated using a polystyrene standard.

2.8. Cell seeding

Cell adhesion and proliferation on the surface of the random and aligned PLGA nanofibers were evaluated to access the viability of Schwann cells. The rat Schwann cell line (CRL-2765) was purchased from ATCC, USA, and was cultured in DMEM (ATCC, USA) supplemented with 10% FBS (Gibco, USA) and 1% penicillin/streptomycin (Himedia, India) and maintained at 37 $^{\circ}\text{C}$ and 5% CO_2 . Prior to cell seeding, each side of the random and aligned PLGA nanofiber mat was sterilized by exposure to UV light for 1 h. Five thousand cells were seeded on each scaffold to study the cell adhesion at various time points and twenty five thousand Schwann cells were seeded to evaluate the cell proliferation.

2.9. Cell adhesion and proliferation

After the first and third days, the scaffolds were rinsed with PBS twice and fixed with 2% glutaraldehyde for 1 h. The scaffolds were further rinsed with distilled water and dehydrated with graded concentrations of alcohol (50%, 75%, 95% and 100%) and were air dried. The dried specimens were

sputter coated with gold and observed under a SEM with an accelerating voltage of 3 kV.

The cell proliferation on both aligned and random scaffolds was determined at different time points 1, 3, 5 and 7 days using a colorimetric MTS assay (CellTiter 96[®] AQueous One solution, Promega, USA). At each time point, the scaffolds were washed with PBS twice and 1 mL of fresh serum-free medium and 200 μ L of MTS reagent were added and incubated for 2 h in a CO₂ incubator at 37 °C. After 2 h, 250 μ L of the SDS reagent was added to stop the reaction. The absorbance was measured at 490 nm using a Tecan microplate reader (Tecan infinite M200, Australia). The absorbance was converted into cell number using a standard curve. Tissue culture polystyrene (TCPS) was used as control.

2.10. Statistical analysis

All values were statistically analyzed and expressed as mean $\pm$ standard deviation. One way analysis of variance (one-way ANOVA) was performed to assess the effect of two different scaffolds on scaffold properties such as pore size, porosity, mechanical strength and wettability. Two-way analysis of variance (two-way ANOVA) was used to evaluate the effect of two different scaffolds and time points on the degradation rate as well as on the cell proliferation rate. In both cases the statistical significance was evaluated at 95% confidence limits ($p < 0.05$). If statistically significant, a post-hoc Tukey test was performed to determine which means were different from the others.

3. Results

3.1. Surface morphology of random and aligned nanofibers

In this study, we have systematically optimized the various process parameters such as flow rate, applied voltage and tip–target distance, and the solution parameters (viscosity, molecular weight, solution conductivity) to obtain defect-free random fibers of PLGA. The surface morphology of PLGA nanofibers (figure 2) was smooth and the diameter of PLGA nanofibers was 197 ± 72 nm. For the fabrication of 3D aligned nanofibers, the speed of rotating mandrel was optimized for PLGA while keeping the other parameters such as concentration, flow rate, needle gauge, applied voltage and tip–target distance constant and the speed of the mandrel was varied from 1000 to 2500 rpm for maximum orientation.

At 1000 rpm, the randomness of the fiber orientation was apparent (figures 3(A1) and (A2)) indicating that 1000 rpm was not sufficient to orient the fibers axially. With the increase in speed to 1500 rpm (figures 3(B1) and (B2)) most of the fibers were in a diagonal direction which confirmed that the speed influences the orientation of fibers. At 2000 rpm, most of the PLGA fibers showed high-order axial alignment (figures 3(C1) and (C2)). However, at higher speeds (2500 rpm) multiple breakages in the collected fibers were observed thereby disrupting the orientation (figures 3(D1) and (D2)). Therefore, 2000 rpm was selected as an ideal speed for PLGA to achieve maximum longitudinal orientation. The mean diameter of the aligned PLGA was found to be

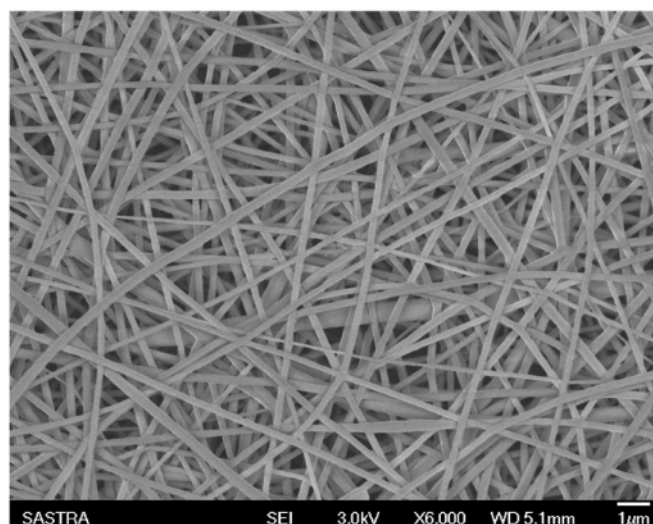


Figure 2. Scanning electron micrograph of two-dimensional random PLGA. Defect-free fibers with average diameter of 192 ± 72 nm were formed.

Table 1. Mechanical properties of random and aligned electrospun scaffolds. Aligned PLGA scaffolds have significantly higher elongation and lower ultimate tensile strength and Young's modulus ($p < 0.05$).

Materials	Ultimate tensile strength (MPa)	Young's modulus (MPa)	Elongation (%)
Random PLGA	$2.6 \pm 0.3^*$	$134.5 \pm 22.7^*$	$14.5 \pm 4.6^*$
Aligned PLGA	$2.1 \pm 0.4^*$	$40.4 \pm 18.2^*$	$26.0 \pm 9.6^*$

187 ± 121 nm. The 2D random and 3D aligned fibers of PLGA were used for further characterization.

3.2. Contact angle

To study the wettable property of the scaffold, we have measured the water contact angle using a goniometer. The random PLGA nanofibers showed the contact angle of about $99.22 \pm 3.6^\circ$, whereas the contact angle of aligned fibers was $112.40 \pm 2.0^\circ$.

3.3. Mechanical testing

The ultimate tensile strength, percentage elongation and Young's modulus were calculated and shown in table 1 for 2D random and 3D aligned nanofibers. The random PLGA nanofibers exhibited significantly higher ultimate tensile strength, higher Young's modulus and lower elongation percentage than aligned PLGA ($p < 0.05$). However, the lower Young's modulus and higher percentage elongation of aligned PLGA proved its higher flexibility and elastic property than random PLGA.

3.4. Porosity and pore size distribution

The porosity of 2D random and 3D aligned nanofibers was determined using a mercury porosimeter (table 2). The

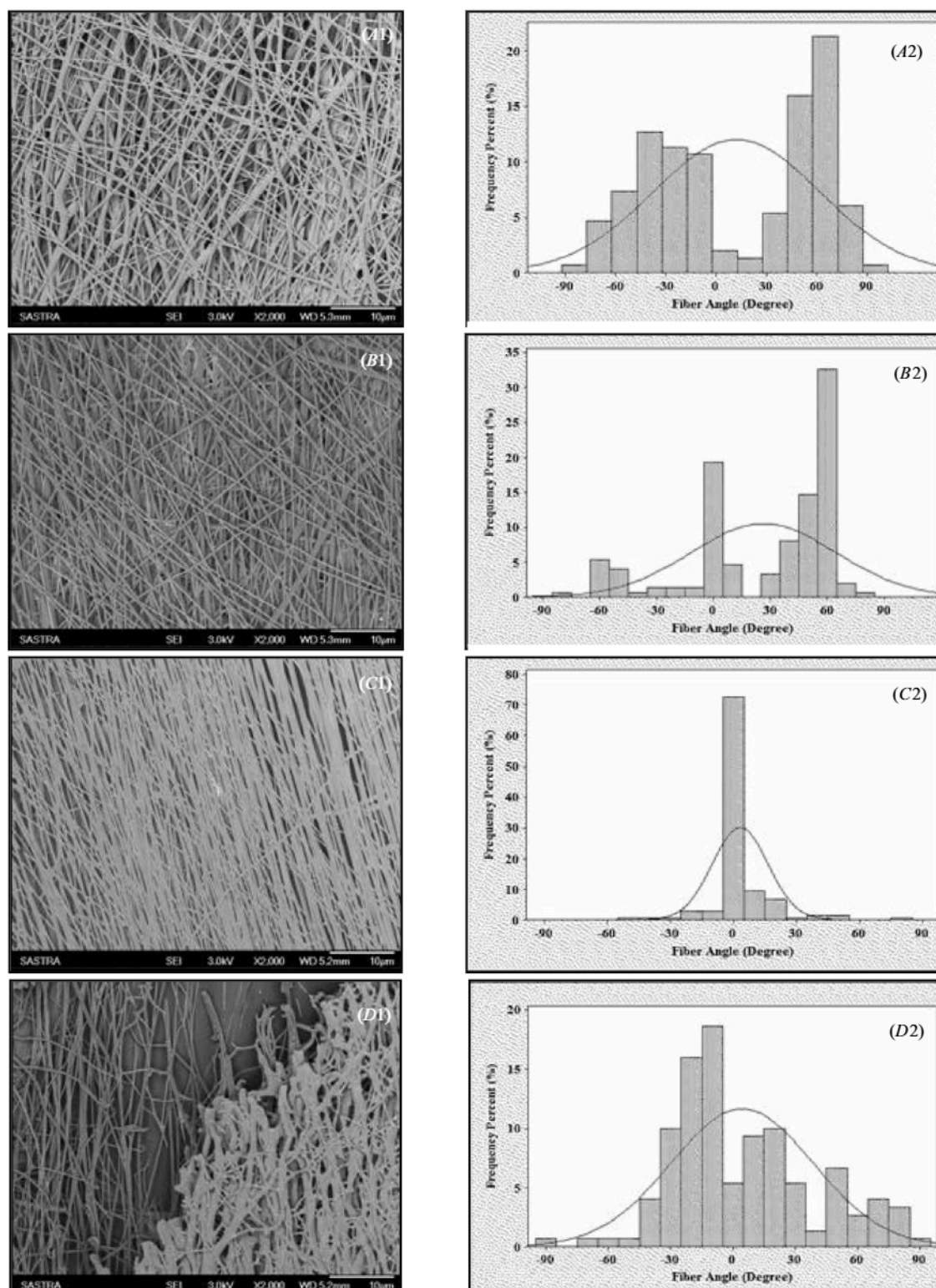


Figure 3. Scanning electron micrographs showing the orientation and histograms of the fiber angle of the PLGA nanofiber at various rotation speeds (A) 1000 rpm; (B) 1500 rpm; (C) 2000 rpm; and (D) 2500 rpm.

porosity of the random and aligned scaffolds was compared ($p > 0.05$). Mercury porosimeter analysis demonstrated that the pore size of random PLGA ($8.0 \pm 2.0 \mu\text{m}$) nanofibers was significantly higher than that of the aligned PLGA ($3.5 \pm 1.1 \mu\text{m}$) ($p < 0.05$).

3.5. *In vitro* degradation

The *in vitro* degradation of both 2D random and 3D aligned electrospun fibers was carried out in PBS at pH 7.4 and at 37 °C with constant shaking for 5 weeks. At the end of

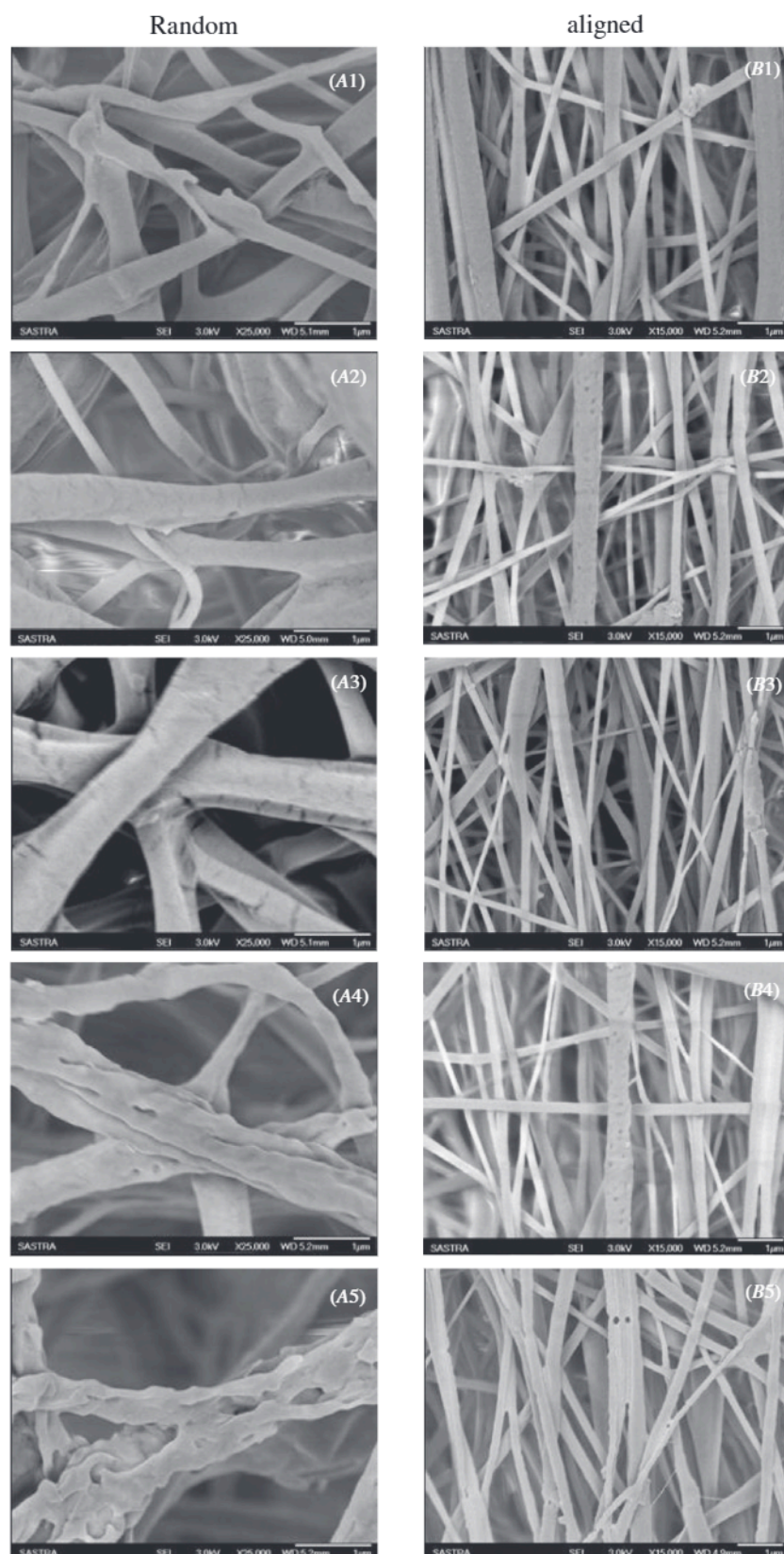


Figure 4. *In vitro* degradation of 2D random of PLGA after (A1) 1 week, (A2) 2 weeks, (A3) 3 weeks, (A4) 4 weeks and (A5) 5 weeks; and 3D aligned PLGA nanofibers after (B1) 1 week, (B2) 2 weeks, (B3) 3 weeks, (B4) 4 weeks and (B5) 5 weeks.

each degradation period, the samples were characterized for morphological change using a SEM and percentage mass loss and percentage molecular weight loss. There was no

apparent change in the morphology of 2D PLGA random fibers up to 3 weeks (figures 4(A1)–(A3)). After 4 weeks, there was presence of tiny pores on the nanofiber surface

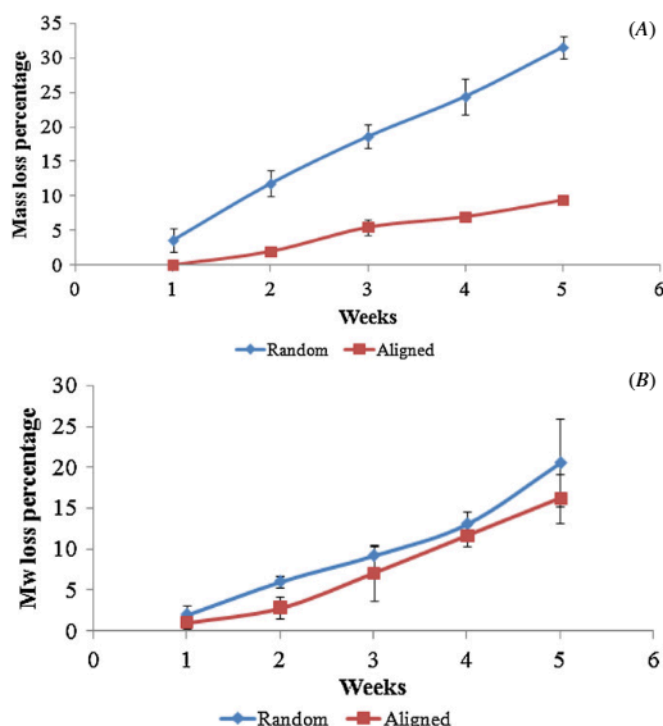


Figure 5. *In vitro* degradation of 2D random and 3D aligned nanofibers: (A) percentage mass loss percentage and (B) molecular weight loss percentage.

Table 2. Percentage porosity, pore size distribution and contact angle for random and aligned electrospun scaffolds ($p < 0.05$).

Electrospun scaffolds	Porosity (%)	Pore size (μm)	Contact angle ($^{\circ}$)
Random PLGA	74.9 ± 10.3	$8.3 \pm 2.0^*$	$99.2 \pm 3.6^*$
Aligned PLGA	75.7 ± 3.2	$3.5 \pm 1.1^*$	$112.4 \pm 2.0^*$

(figures 4(A4) and (A5)) indicating the onset of degradation of PLGA fibers. It was apparent that the pore size increased with the time indicating the bulk degrading behavior of random PLGA. Interestingly, aligned PLGA fibers did not show any morphological change up to 4 weeks (figures 4(B1)–(B4)). Only after 5 weeks, there was a visible thinning of fibers (figure 4(B5)), confirming the slow degradation rate of the aligned PLGA scaffold. This was also confirmed by determining the mass loss percentage. The PLGA random fibers showed significantly greater mass loss percentage (31.5%) after 5 weeks than the mass loss percentage of the aligned PLGA scaffold ($p < 0.05$) (figure 5(A)). At the end of 5 weeks, random PLGA nanofibers were found to have significantly higher molecular weight loss percentage when compared to aligned PLGA nanofibers ($p < 0.05$) (figure 5(B)).

3.6. Cell adhesion and proliferation

The morphology of Schwann cells was observed using a SEM after the first and third days. After 1 day cell culture, the cells on the aligned fibers (figure 6(A1)) follow the fiber direction, whereas the random fibers (figure 6(B1)) showed randomly oriented cells. After 3 days, cells on aligned

fibers (figure 6(A2)) acquired spread morphology and covered the maximum surface of the nanofibers than random fibers (figure 6(B2)). The cell proliferation of Schwann cells on both random and aligned nanofibers was quantitatively evaluated. It was observed that significantly higher number of cells were found on the aligned scaffold when compared to random and TCPS (control) (figure 7).

4. Discussion

Nerve tissue is composed of bundles of nerve fibers packed together by connective tissue to transmit the signal unidirectionally [21]. Moreover, across a smaller nerve gap, the regeneration is promoted by the formation of a fibrin cable which in turn orients the Schwann cells longitudinally [6]. Electrospinning has been used to develop longitudinally oriented 3D nanofibers to bridge larger gaps, resemble the native architecture of ECM and orient the axons across the lesion. In this study, we have optimized the process parameters such as flow rate, applied voltage and tip–target distance, and the solution parameters such as viscosity, molecular weight and solution conductivity to fabricate defect-free random and aligned fibers of PLGA. The choice of the solvent, concentration of polymers, flow rate, tip–target distance, and needle diameter will greatly influence the fiber size and morphology [11].

Figure 1 illustrates the electrospinning setup (figure 1(A)) used for fabricating a tubular scaffold (figure 1(B)) with longitudinally oriented nanofibers. It is basically the same as the usual setup except for the use of a rotating metal rod comprising a number of insulating gaps of 1.5 cm in its middle as a collector. Such a collector is fabricated simply by introducing an insulating sleeve into the metal rod. After applying high voltage, the electric field in close proximity to the collector was split into two portions pointing toward the opposite edges of the insulating gap [22]. It is assumed that the charged fibrous jet ejected from the needle experienced electrostatic forces. First electrostatic force is used to pull the charged fibers toward the target. Due to Coulombic interactions, the charged fiber in the vicinity of each electrode creates another strongest electrostatic force used to stretch the nanofibers across the insulating gap in the rod [23]. The major advantage of this setup is that the fibers deposited across the gap can slowly lose their charge which can create an electrostatic repulsion between the collected fibers and the depositing fibers for few seconds. This repulsive force further improves the orientation of fibers. Thereby we were fabricated tubes made of longitudinally aligned fibers by varying the speed of the rotating collector.

In this study, we have used a dynamic collector to generate highly oriented fibers by varying the rotation rate. Similar to the findings of Zussman *et al*, we observed that the rotating speed of the metal rod influenced the fiber orientation for PLGA [15, 24]. The axial deposition of fibers onto the rotating rod is mainly through stretching of fibers across the insulating gap. The wettability of the scaffold was increased in aligned rather than in random fibers. The increase in the contact angle of axial fibers may be due to the decreased pore size having direct influence on smoothness of the scaffolds.

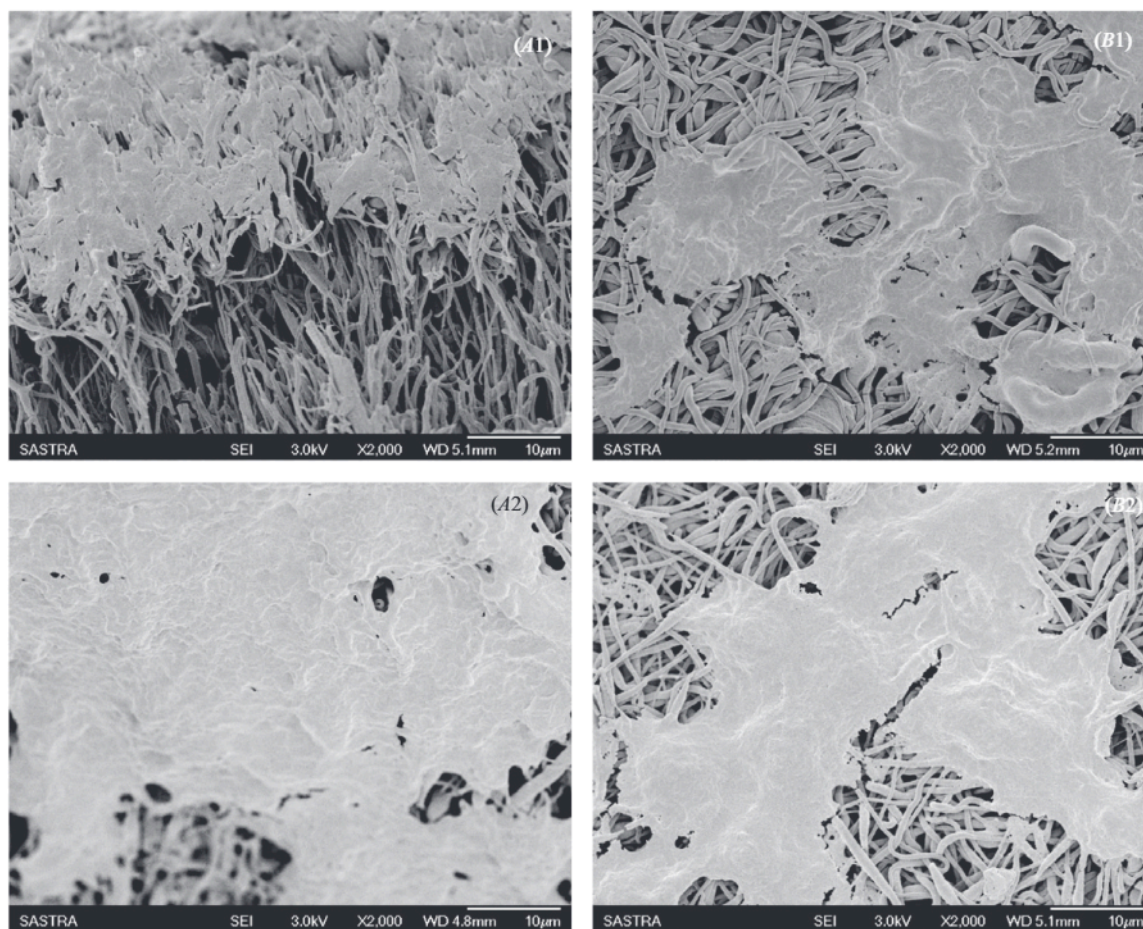


Figure 6. Scanning electron micrograph of Schwann cells on scaffolds after (A1) first day on aligned PLGA, (B1) first day on random PLGA, (A2) third day on aligned PLGA and (B2) third day on random PLGA.

The mechanical properties of 2D random and 3D axially aligned electrospun fibers were measured using a uniaxial testing machine with a deforming speed of 1 mm s^{-1} at room temperature to evaluate the effect of fiber alignment and materials on mechanical strength. The tensile strength of the PLGA of random fibers was significantly greater than that of the aligned PLGA fibers. This is because the entwined fibers in the random scaffold offer more surface resistance, thereby improving the tensile strength than aligned fibers. However, the tensile strength of the aligned PLGA scaffold (2.1 MPa) closely matches with the tensile strength (1.4 MPa) of acellular rat peripheral nerve [25]. The significantly lower Young's modulus and greater elongation percentage of aligned fibers demonstrated the flexibility of aligned scaffolds than that of random scaffolds [26].

Porosity is an important characteristic of the scaffolds in tissue engineering since it provides structural space for cell lodging, exchange of nutrients and metabolic waste and angiogenesis [27]. Yang *et al* fabricated nanostructured porous PLLA scaffolds for nerve tissue engineering and observed that random orientation increased the pore size in the nanofibers as compared to aligned fibers [28]. In the present study, a significant reduction in the pore size was observed in axially aligned PLGA fibers as compared to random fibers. This may be attributed to the fact that the axial alignment improves the compactness of the fibers through the speed of the rod,

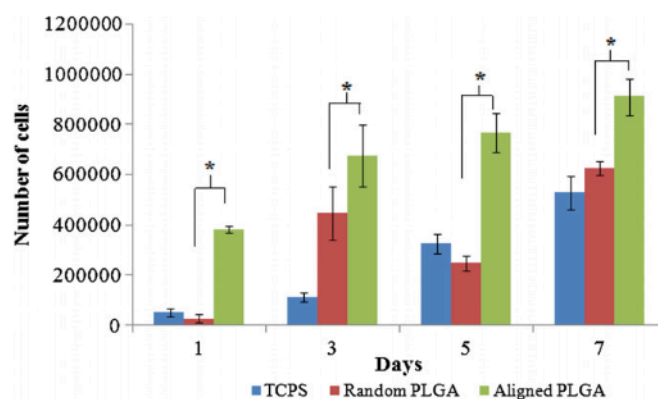


Figure 7. Number of Schwann cells on the surface of random PLGA, aligned PLGA and TCPS (control) (* $p < 0.05$).

thereby reducing the pore size [29]. Gupta *et al* reported that the $1\text{--}2 \mu\text{m}$ pore size of the scaffold was found to be desirable for cell migration and nutrient flow [26]. The aligned scaffold showed suitable pore size ($3.5 \mu\text{m}$) for cell supporting capacity which is close to the range of $1\text{--}2 \mu\text{m}$. The scaffold with low degradation behavior and swelling property acquires more attention to withstand nerve compression [26]. In this study, we have observed the influence of longitudinal orientation on the manipulation of degradation behavior. The significant reduction in the degradation in axial fibers rather than in

random 2D fibers may be due to the decreased pore size of the aligned fiber which decreases the diffusion rate of PBS thereby controlling the degradation rate.

Schwann cells are the glial cells present in the PNS that surround the neuronal axons to form a myelin sheath and helps in aiding the axonal regeneration by providing neurotropic factors and cell adhesion molecules [30, 31]. A two-dimensional aligned PCL/gelatin nanofibrous scaffold fabricated by Gupta *et al* showed lesser proliferation of Schwann cells than random fibers [26]. Currently three-dimensional constructs have received more attention to maximize the topographical directional cue for neurite outgrowth and to minimize the dead space, cross-sectional area occupied by the polymer [14]. In our study, Schwann cells were adhered on the surface of the aligned 3D PLGA fibers and the cell proliferation study confirmed that the cells on the aligned fibers have a significantly higher proliferation than those on random and TCPS. This observation could be attributed to the fact that the orientated as well as the three-dimensional architectures improve the arrangement of cells which assists the proliferation of a higher number of cells. SEM micrographs of Schwann cells after days 1 and 3 on aligned scaffolds supported the same by covering a maximum surface of the scaffolds. These results indicated that the fabricated oriented scaffolds have better flexibility and prolonged degradation behavior with lesser pore size than the random scaffolds. The aligned scaffolds were also able to guide the neural cells along their length and may provide enough physical substrate to enable regenerating fibers to traverse the larger gaps.

5. Conclusions

We have established a novel approach to producing a tubular scaffold with longitudinally aligned structure by modifying the electrospinning process. Such a technique also has an advantage of developing a thicker layer of oriented fibers. In this study, PLGA of both random and longitudinally oriented nanofibers was fabricated successfully through electrospinning. It was confirmed that the orientation of the fiber plays a major role in the tailoring of the scaffold properties such as porosity, mechanical property, degradability and wettability. The longitudinally oriented scaffold demonstrated good flexibility, slower degradation, less pore size with comparable porosity of random fibers and a better cell proliferation rate than its random fibers. In addition to this, the similar topography of natural ECM indicates that the longitudinally aligned scaffold is a suitable substitute for orienting the neural cells across the lesion.

Acknowledgment

The authors wish to acknowledge the infrastructural grant provided by the Nano Mission Council, DST (00/IFD/5634/2006–7) and financial assistance provided by the Nano Science & Technology Initiative, DST, Govt of India (35/12/2009-NAN/BMS).

References

- [1] Subramanian A, Krishnan U M and Sethuraman S 2009 Review. Development of biomaterial scaffold for nerve tissue engineering: biomaterial mediated neural generation *J. Biomed. Sci.* **16** 108
- [2] Schmidt C E and Leach J B 2003 Neural tissue engineering: strategies for repair and regeneration *Annu. Rev. Biomed. Eng.* **5** 293–347
- [3] Wang S, Wan A C A, Xu X, Gao S, Mao H–Q, Leong K W and Yu H 2001 A new nerve guide conduit material composed of a biodegradable poly(phosphoester) *Biomaterials* **22** 1157–69
- [4] Zhang L and Webster T J 2009 Review. Nanotechnology and nanomaterials: promises for improved tissue regeneration *Nanotoday* **4** 66–80
- [5] Filbin M T, Qiu J and Cai D 2001 Glial influences on axonal regeneration *Glial Cell Development—Basic Principles and Clinical Relevance* vol 14 ed K R Jessen and W D Richardson (Oxford: Oxford University Press) pp 279–98
- [6] Bellamkonda R V 2006 Peripheral nerve regeneration: an opinion on channels, scaffolds and anisotropy *Biomaterials* **27** 3515–8
- [7] Schnell E, Klinkhammer K, Balzer S, Brook G, Klee D, Dalton P and Mey J 2007 Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly- ϵ -caprolactone and a collagen/poly- ϵ -caprolactone blend *Biomaterials* **28** 3012–25
- [8] Li W J, Laurencin C T, Caterson E J, Tuan R S and Ko F K 2002 Electrospun nanofibrous structure: a novel scaffold for tissue engineering *J. Biomed. Mater. Res.* **A 60** 613–21
- [9] Kumbar S G, James R, Nukavarapu S P and Laurencin C T 2008 Electrospun nanofibers scaffolds: engineering soft tissues *Biomed. Mater.* **3** 034002
- [10] Jing A, Zhang H, Zhang J, Zhao Y and Yuan X 2009 Preparation and antibacterial activity of electrospun chitosan/poly(ethylene oxide) membranes containing silver nanoparticles *Colloid Polym. Sci.* **287** 1425–34
- [11] Dhandayuthapani B, Krishnan U M and Sethuraman S 2010 Fabrication and characterization of chitosan–gelatin blend nanofibers for skin tissue engineering *J. Biomed. Mater. Res. B* **94** 264–72
- [12] Chew S Y, Mi R, Hoke A and Leong K W 2008 The effect of the alignment of electrospun fibrous scaffolds on Schwann cell maturation *Biomaterials* **29** 653–61
- [13] Bini T B, Gao S, Wang S and Ramakrishna S 2006 Poly(L-lactide-co-glycolide) biodegradable microfibers and electrospun nanofibers for nerve tissue engineering: an *in vitro* study *J. Mater. Sci.* **41** 6453–9
- [14] Kim Y, Haftel V K, Kumar S and Bellamkonda R 2008 The role of aligned polymer fiber-based constructs in bridging of long peripheral nerve gaps *Biomaterials* **29** 3117–27
- [15] Teo W E, Kotaki M, Mo X M and Ramakrishna S 2005 Porous tubular structures with controlled fibre orientation using a modified electrospinning method *Nanotechnology* **16** 918–24
- [16] Burger C, Hsiao B S and Chu B 2006 Nanofibrous materials and their applications *Annu. Rev. Mater. Res.* **36** 333–68
- [17] Chew S Y, Wen J, Yim E K F and Leong K W 2005 Sustained release of proteins from electrospun biodegradable fibers *Biomacromolecules* **6** 2017–24
- [18] Teo W E and Ramakrishna S 2006 A review on electrospinning design and nanofibre assemblies *Nanotechnology* **17** R89–106
- [19] Theron A, Zussman E and Yarin A L 2001 Electrostatic field-assisted alignment of electrospun nanofibres *Nanotechnology* **12** 384–90

- [20] Bhang S H, Lim J S, Choi C Y, Kwon Y K and Kim B-S 2007 The behavior of neural stem cells on biodegradable synthetic polymers *J. Biomater. Sci., Polym. Ed.* **18** 223–39
- [21] Hagiwara S and Tasaki I 1958 A study on the mechanism of impulse transmission across the giant synapse of the squid *J. Physiol.* **143** 114–37
- [22] Teo W E and Ramakrishna S 2005 Electrospun fibre bundle made of aligned nanofibers over two fixed points *Nanotechnology* **16** 1878–84
- [23] Li D, Wang Y and Xia Y 2003 Electrospinning of polymeric and ceramic nanofibers as uniaxially aligned arrays *Nano Lett.* **3** 1167–71
- [24] Zussman E, Rittel D and Yarin A L 2003 Failure modes of electrospun nanofibers *Appl. Phys. Lett.* **82** 3958–60
- [25] Borschel G H, Kia K F, Kuzon W M and Dennis R G 2003 Mechanical properties of acellular peripheral nerve *J. Surg. Res.* **114** 133–9
- [26] Gupta D, Venugopal J, Prabhakaran M P, Giri Dev V R, Low S, Choon A T and Ramakrishna S 2009 Aligned and random nanofibrous substrate for the *in vitro* culture of Schwann cells for neural tissue engineering *Acta Biomaterialia* **5** 2560–9
- [27] Nair L S, Bhattacharya S and Laurencin C T 2008 Nanotechnology and tissue engineering: the scaffold based approach *Nanotechnol. Life Sci.* **8** 1–65
- [28] Yang F, Murugan R, Ramakrishna S, Wang X, Ma Y X and Wang S 2004 Fabrication of nano-structured porous PLLA scaffold intended for nerve tissue engineering *Biomaterials* **25** 1891–900
- [29] Mobarakeh L G, Prabhakaran M P, Morshed M, Nasr-Esfahani M H and Ramakrishna S 2008 Electrospun poly(3-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering *Biomaterials* **29** 4532–9
- [30] Ide C 1996 Peripheral nerve regeneration *Neurosci. Res.* **25** 101–21
- [31] Bunge R P 1993 Expanding roles for the Schwann cell: ensheathment, myelination, trophism and regeneration *Curr. Opin. Neurobiol.* **3** 805–9

