Published in final edited form as:

J Biomed Mater Res A. 2015 February ; 103(2): 593–603. doi:10.1002/jbm.a.35208.

Electrospinning of unidirectionally and orthogonally aligned thermoplastic polyurethane nanofibers: Fiber orientation and cell migration

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Abstract

Unidirectionally and orthogonally aligned thermoplastic polyurethane (TPU) nanofibers were electrospun using a custom-built electrospinning device. The unidirectionally aligned fibers were collected using two parallel copper plates, and the orthogonally aligned fibers were collected using two orthogonal sets of parallel copper plates with alternate negative connections. Carbon nanotubes (CNT) and polyacrylic acid (PAA) were added to modify the polymer solution. It was found that both CNT and PAA were capable of increasing solution conductivity. The TPU/PAA fiber showed the highest degree of fiber orientation with more than 90% of the fibers having an orientation angle between -10° and 10° for unidirectionally aligned fibers, and for orthogonally aligned fibers, the orientation angle of 50% fibers located between -10° and 10° and 48% fibers located between 80° and 100° . Viability assessment of 3T3 fibroblasts cultured on TPU/PAA fibers suggested that the material was cytocompatible. The cells' orientation and migration direction closely matched the fibers' orientation. The cell migration velocity and distance were both enhanced with the guidance of fibers compared with cells cultured on random fibers and common tissue culture plastic. Controlling cell migration velocity and directionality may provide ways to influence differentiation and gene expression and systems that would allow further exploration of wound repair and metastatic cell behavior.

Keywords

electrospinning; fiber orientation; thermoplastic polyurethane; fibroblast; cell migration

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Additional Supporting Information may be found in the online version of this article.

INTRODUCTION

Electrospinning is a versatile method for the production of uniform, continuous fibers with diameters ranging from the nanoscale to the microscale.¹ Electrospinning is one of the most popular techniques for tissue engineering scaffold fabrication due to its capability to produce porous fiber membranes with high surface-to-mass ratios that facilitate cell incorporation and nutrition perfusion.² In electrospinning, typically, a stream of polymer solutions supplied by a syringe pump is connected to a high electric field. The electric field provides an electric force to the polymer solution resulting in the formation of fibers as the electric field surpasses a threshold value where electrostatic repulsion forces from surface charges overcome the surface tension.^{3,4} One challenge in the electrospinning process arises around controlling the deposition of the fibers, since the as-spun fibers are nonwoven because of the bending instability of the highly charged solution jet.⁵ In tissue engineering, these random nonwoven fibers can be used to mimic the structure of certain native extracellular matrices (ECMs). However, aligned fibers featuring an aligned structure similar to such tissues as muscles and tendons are also useful for guiding cell growth with the desired anisotropy.⁶ For example, electrospun aligned nanofibers provide beneficial topographical cues that enhance the extension and growth of osteoblasts,⁷ cardiac myocytes,⁸ vascular cells,⁹ neural cells,¹⁰ and skeletal muscle cells.¹¹

Various methods have been reported for the preparation of macroscopically aligned nanofibers by employing a special fiber collecting system, such as a high-speed rotating drum,¹² copper wire drum,¹³ scanning tip,¹⁴ or parallel conductive plates.¹⁵ Among them, the most convenient method utilizes conductive plates with an insulating gap as the collector. It has been reported that the insulating gap size affects the degree of fiber orientation dramatically—namely, larger gap sizes result in better fiber alignment.¹⁶ Aligned fibers of various materials have been obtained using this method, such as polyethylene oxide,¹⁷ polycaprolactone (PCL),¹⁸ poly(vinyl pyrrolidone),¹⁵ poly(vinyl alcohol),¹⁹ poly(lactic-co-glycolic acid),²⁰ and polyamide-6 (PA-6 or Nylon-6).²¹ However, the alignment achieved by this method was not high enough to direct cell migration, particularly for nonpolar polymers. The solution conductivity could be another crucial factor that affects the fiber orientation. It was reported that addition of a conductivity-enhancing salt (e.g., benzyl triethylammonium chloride) could improve poly (hydroxybutyrate-co-hydroxyvalerate) fiber alignment using a rotating drum collector.²² Similarly, the addition of sodium chloride (NaCl) has been reported to affect polyamide (PA) solution conductivity and fiber formation significantly.²³ Hence, these results suggest that the conductivity of a solution plays an important role in electrospinning.

In addition to producing unidirectionally aligned fibers, attempts have been made to guide fiber orientation in various directions in order to obtain anisotropic fibrous membranes. Fibers with a defined orientation are useful in applications such as conductive electrodes,²⁴ selective filtration,^{25,26} and direct cell behavior.²⁷ One method has been reported that uses layer-by-layer stacking films of aligned fibers.²⁸ Another method incorporates rotation of the collection substrate to obtain grid-patterned fibers.¹⁹ Using alternating negative connections of two pairs of parallel conductive plates in an orthogonal configuration to collect fibers could be a more efficient approach to guiding fiber orientation. This method

was investigated in this study. The highly ordered fibers might be useful in electrical and optical applications as well as in tissue engineering by directing cellular behavior.

Thermoplastic polyurethane (TPU) is a type of flexible biodegradable elastomer that offers high elongation, moderate tensile strength and Young's modulus, and excellent abrasion and tear resistance.²⁹ TPU belongs to the polyurethane (PU) family and is widely commercially available. It has extensive potential to be used in soft tissue engineering applications such as in wound dressings³⁰ and vascular grafts.³¹ Although aligned PU³² and PU composite (e.g., PU/carbon nanotube (CNT)³³ and TPU/chitosan/collagen³⁴ electrospun fibers have been prepared using a rotation drum as the collector, the extent of fiber orientation had a large deviation which might not have been high enough to direct cell migration. Moreover, to the best of our knowledge, no studies have been published that incorporate conductive plates to produce aligned or orthogonal TPU electrospun fibers. Therefore, it is highly beneficial to investigate methods to improve TPU electrospun fiber orientation and the cellular response to such fibers.

In this study, unidirectionally and orthogonally aligned TPU nanofibers were collected using two parallel copper plates and two pairs of parallel copper plates perpendicular to each other, respectively. Additives such as CNT and polyacrylic acid (PAA) were added into the TPU solution to improve the fiber orientation. Cell viability and cell migration on the unidirectionally and orthogonally aligned nanofibers were studied by 3T3 fibroblast cell culture.

MATERIALS AND METHODS

Materials

Medical grade thermoplastic polyurethane (Texin Rx85A) was supplied by Bayer Corp. PAA and dimethylformamide (DMF) were purchased from Sigma-Aldrich. All of the above materials were used as received. Multiwall CNTs were purchased from the Chinese Academy of Sciences, Chengdu Organic Chemistry Carboxylation of CNT was performed in order to improve the interaction between CNT and the polymer matrix. The CNTs were dispersed into 98% sulfuric acid at a concentration of 10 mg/40 mL and treated with a bath sonicator (Fisher Scientific) for 3 h. Then the solution was diluted and washed excessively with deionized water until no residual acid was present, followed by filtering through a PTFE filter.³⁵

Solution preparation

The TPU solution was prepared by dissolving 2 g of TPU pellets into 20 mL of DMF at 70° C for 8 h with 300 rpm magnetic stirring. The TPU/CNT solution was prepared by ultrasonically dispersing 20 mg of CNT (1% wt of TPU) into 20 mL of DMF for 1 h using a probe ultrasonic device (UP200H, Hielscher Ultrasound Tech.), then dissolving 2 g of TPU pellets in the solution at 70° C for 8 h with 300 rpm magnetic stirring. The TPU/PAA solution was prepared by dissolving 2 g of TPU pellets and 20 mg of PAA (1% wt of TPU) into 20 mL of DMF at the aforementioned conditions.

Electrospinning process

The electrospinning process was carried out using a custom-built electrospinning device. The prepared solution was loaded in a plastic syringe connected to an 18-gauge blunt-end needle then mounted on a digital syringe pump (Harvard Bioscience Company). The electrospinning procedure was carried out using 18 kV voltage, a 150 mm needle-to-target distance, and a 0.5 mL/h flow rate for 40 min. The setups used to collect unidirectionally and orthogonally aligned fibers are illustrated in Figure 1(a). The unidirectionally aligned fibers were collected using two parallel copper plates ($50 \times 30 \times 1.5 \text{ mm}^3$) with a 3 cm distance between them [Fig. 1(a-i)]. The orthogonally aligned fibers were collected using four orthogonal copper plates ($30 \times 30 \times 1.5 \text{ mm}^3$) with a 4 cm distance between each pair [Fig. 1(a-ii)], with the negative connection on one pair of the parallel plates at a time and switched every 10 min, such that the connection switched four times during 40 min of electrospinning. The effect of plate distance on the fiber diameter was investigated using different plate distances.

3T3 Fibroblast cell culture

The electrospun fibers were wrapped on autoclave-sterilized stainless steel washers (McMaster–Carr) then sterilized using UV light for 30 min on each side of the sample. Samples were placed in 24-well tissue culture-treated polystyrene plates. 3T3 cells were treated with ethylenediaminetetraacetic acid (Life Technologies) for 5 min and washed with phosphate-buffered saline (PBS) prior to seeding. The cell seeding density for live/dead tests was $1.25 \times 10^5 \text{ cells/cm}^2$, and it was $1.25 \times 10^3 \text{ cells/cm}^2$ for cell migration tests. 3T3 cells were fed regularly with a 20% fetal bovine serum (FBS) media for both routine maintenance and TPU assessment specimens. This media consisted of 80% high-glucose Dulbecco's modified eagle medium (Life Technologies), with 20% FBS (WiCell), 1 unit/mL penicillin (Life Technologies), 1 $\mu\text{g/mL}$ streptomycin (Life Technologies), and 2 mM L-glutamine (Life Technologies). Spent medium was aspirated and replaced with 1 mL of fresh medium daily for screening samples.

Characterizations

Solution conductivity and pH—The conductivity and pH of the solutions prepared for electrospinning were measured at room temperature using a pH/conductivity (AP85 Portable, Fisher Scientific). The pH meter was calibrated with 3 pH buffer (pH = 4.1, 7, 10) prior to use. The conductivity meter was calibrated to the 0.0–19.9 μS range prior to use. The results were the average value of three different measurements.

Solution viscosity—The complex viscosity of the solutions prepared was tested via a parallel-plate rheometer (AR 2000ex, TA). A 25-mm parallel-plate geometry was used and all tests were performed at 25° C. Oscillatory frequency sweep tests were performed at a constant stress of 1 Pa with an increase of angular frequency from 0.1 to 100 rad/s.

X-ray photoelectron spectroscopy—X-ray photoelectron spectroscopy (XPS) C1 core-level signals were analyzed to identify the proportion of carbon bonds in the samples. XPS measurements were performed on an X-ray photoelectron spectrometer with a focused, monochromatic K-alpha X-ray source and a monoatomic/cluster ion gun (Thermo

Scientific). The spectra were Gaussian fitted and the proportion of each bond was determined from the peak area ratios.

Raman spectra—Raman spectra analyses of the scaffolds were performed with a DXR Raman microspectrometer (Thermo Scientific). The electrospun fiber mats were folded and fixed on glass slides and CNT powder was directly placed on each glass slide for measurements. Raman spectra were recorded in the range of 100 to 3500 cm^{-1} .

Scanning electron microscopy—The morphology of the electrospun membranes was observed using scanning electron microscopy (SEM). The electrospun membranes were wrapped on a cover slip and coated with a thin film of gold for 40 s. The samples were observed on a fully digital LEO GEMINI 1530 SEM (Zeiss, Germany) at a voltage of 3 kV. The orientation angle and diameter of the fibers were measured from SEM images using the Image Pro-Plus software. The angle between fibers and the vertical direction was measured as the fiber orientation angle; 50 fibers were measured for each sample. The fiber diameter was the average value of at least 50 fibers.

Cell viability assay—Fibroblast viability was determined 3 days and 10 days after cell seeding. Viability was assessed via a Live/Dead Viability/Cytotoxicity Kit (Invitrogen). The stain utilized green fluorescent Calcein-AM to target esterase activity within the cytoplasm of living cells, and red fluorescent ethidium homodimer-1 (EthD-1) to indicate cell death by penetrating damaged cell membranes. Stained cells were imaged with a Nikon A1RSi inverted confocal microscope.

Cell fixation for SEM—The samples used for cell viability testing were rinsed twice with Hanks' balanced salt solution (Thermo Scientific). HyClone HyPure molecular biology grade water (Thermo Scientific) was mixed with paraformaldehyde to make a 4% solution. The rinsed samples were then immersed in the fixing solution for 30 min. The samples were dehydrated using a series of ethanol washes (50%, 80%, 90%, and 100% for 30 min each), and finally dried in a vacuum desiccator for 2–3 h before gold sputtering for SEM.

Cytoskeleton study—The cell cytoskeleton organization was determined by phalloidin-tetramethylrhodamine B isothiocyanate (phalloidin-TMRho, Sigma) staining. Cells were first fixed in 4% paraformaldehyde (EMS) diluted in PBS for 15 min at room temperature. They were then washed with PBS and permeabilized with 0.1% Triton-X in PBS for 5 min at room temperature. The cells were washed one more time and treated with 0.3 μM phalloidin-TMRho with DAPI for 1 h at room temperature. Last, samples were washed with PBS and immediately imaged using the same confocal microscope mentioned above.

MTS (cell proliferation) assay—CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) was used to determine the number of cells on the electrospun fibers. Standard curves were established by performing the tests on cells seeded on the cell culture wells without scaffolds and confirmed by comparison to hemocytometer readings prior to these experiments. Upon testing, cells were treated with an 83% media, 17% MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) solution and allowed to incubate for exactly 1 h. After incubation, 100 μL of spent media

were removed and added to a clear 96-well plate. The absorbance of this plate at the 450 nm wavelength was read with a GloMax-Multi + Multiplate Reader (Promega) and the subsequent number of cells was determined relative to the negative control.

Cell migration—Migration assays were conducted to assess the movement of cells attached to the unidirectionally and orthogonally aligned fibers in comparison to the cells cultured on random fibers and tissue culture plastic (TCP), which are directionally undefined. 3T3 fibroblasts were seeded at a density of 1.25×10^3 cells/cm² onto TPU/PAA unidirectionally and orthogonally aligned fibers and TCP as a control. Cultures were maintained in a hydrated Tokai Hit Microscope Incubation System, which was held at 37° C. A Tokai Hit GM-8000 Gas Mixer was used to provide an environment with 20% CO₂ and 5% O₂. Bright field images were captured every 7.5 min from day 2 to day 5 and then converted to videos.

The positions of nine individual centroids of cells were tracked manually from the images using Image Pro-Plus. If the cell split during the period, only one of the new cells was traced. The start points of each cell were defined at the same position to demonstrate the cell migration trend. At the final state, the cell orientation angles of 50 spindle-shaped cells were measured (the cell orientation angle was the angle between the fiber alignment direction and the axis of the spindle-shaped cell). The cell migration velocity was calculated by dividing the accumulated cell migration distance by time. The Euclidean distance (length of the line segment between the cell start point and the farthest point from the start point) was measured as well to further explain cell migration directionality.

Statistical analysis—Statistical analysis of biological experiments was performed using a one-way analysis of variance and the difference significance between groups was compared with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Solution properties and component analysis

The photographs of solutions prepared for electrospinning are shown in Figure 1(b). The TPU solution was yellow and opaque. The TPU/CNT solution was completely black due to the addition of CNT. The TPU/PAA solution was clear and transparent with a light yellow color, indicating that the addition of PAA could assist in dissolving TPU in DMF. The conductivity of the solution improved significantly after the addition of CNT and PAA into TPU, as indicated by Figure 1(c). The improvement was more significant for the TPU/PAA solution. The enhancement of the TPU/CNT solution conductivity was due to the conductive filler CNT, while, for the TPU/PAA solution, the improvement could be attributed to the ionization of the PAA molecules. The solution pH value, however, showed an inverse trend. This was due to the carboxylation of CNT for the TPU/CNT solution, and the carboxyl groups of PAA for the TPU/PAA solution.

The solutions' complex viscosity results are shown in Figure 1(d), from which it was noticed that at an angular frequency of 1 rad/s, the TPU/CNT solution (737.5 Pa·s) had a higher viscosity than the TPU solution (242.8 Pa·s). This may have been because the CNTs

hindered the movement of the TPU molecular chains in the solution. On the contrary, the TPU/PAA solution (115.7 Pa·s) showed a lower viscosity than the TPU solution (242.8 Pa·s). This might indicate that PAA can not only improve the TPU dissolvment in DMF [Fig. 1(b)], but that it may also plasticize the TPU solution. The viscosity reduction disappeared at high frequencies which might due to the molecular chain entanglement increase. These variations in solution properties (conductivity and viscosity) are supposed to affect the electrospun fiber formation.

The presence of CNT and PAA components in the electrospun fiber mats was verified via XPS and Raman spectra as shown in Figure 2. It was noticed from Figure 2(a) that the C1s peaks of TPU and TPU composites consisted of four bonds³⁶: C—C, C—O, C—N, and C=O. According to the quantitative results of the fitted individual peaks from Figure 2(b), it was found that the C—C bond position of TPU shifted to a lower energy level due to the isolation effect of TPU. The percentage of each bond result showed that the C—C bond proportion was higher for the TPU/CNT sample than the TPU and TPU/PAA samples because the CNT consisted of a lot of C—C bonds. The C=O bonds in the TPU/PAA sample were much higher than in the other samples due to the presence of carboxyl groups in the PAA. The existence of CNT in the TPU/CNT fiber mat was further confirmed by a Raman spectra test as shown in Figure 2(c). All three bands of CNT (G, D, and G') were detected in the TPU/CNT sample as shoulder peaks. The PAA content in the TPU/PAA sample, however, could not be detected using the Raman spectra test. (FTIR) and X-ray diffraction (XRD) could not detect the difference among the samples due to the low sensitivity of the instruments for CNT and PAA (see Supporting Information Figure S1 and S2).

Fiber morphology

The morphologies of unidirectionally and orthogonally aligned fibers electrospun from different solutions are shown in Figures 3 through 5. The morphology of the electrospinning random TPU/PAA nanofibers was shown in the Supporting Information Figure S3. As shown in Figure 3, the neat TPU fibers could form neither unidirectionally aligned fibers using the two plate collector [Fig. 3(a,b)] nor orthogonally aligned fibers using the four plate collector [Fig. 3(c,d)], even though there was some sort of alignment in the fiber orientation. The orientation angle distribution [Fig. 3(e)] showed somewhat of a convergence at 0° for unidirectionally aligned fibers, with 58% of the fibers' orientation angle between -10° and 10°. For orthogonally aligned fibers, 28% of the fibers had orientation angles between -10° and 10°, and 24% of the fibers had orientation angles between 80° and 100°. Many fibers, however, had orientation angles far away from 0° or 90°. This is due to the high flexibility of TPU molecules caused high bending instability and the low conductivity of the TPU solution could not provide enough electric force to align the fibers in the electric field.

The TPU/CNT fiber morphology is shown in Figure 4. It is obvious that TPU/CNT fiber orientation for both unidirectionally and orthogonally aligned fibers were performed better than that of neat TPU as indicated by Figure 4(e). About 60% of the fibers had orientation angles between -10° and 10° for unidirectionally aligned fibers, and for orthogonally aligned fibers, 50% of the fibers had orientation angles between -10° and 10°, and 34% of the fibers had orientation angles between 80° and 100°. Moreover, more fibers were

collected within the same electrospinning period using the TPU/CNT solution than the neat TPU solution, which might have been because more fibers were deposited within the electric field between the plates with the help of the elevated solution conductivity.

The TPU/PAA solution performed even better than the TPU/CNT solution in terms of fiber orientation for both unidirectionally and orthogonally aligned fibers as shown in Figure 5. Most fibers (about 94%) had orientation angles between -10° and 10° for unidirectionally aligned fibers. Likewise, about 50% of the fibers had orientation angles between -10° and 10° , and 48% of the fibers had orientation angles between 80° and 100° for orthogonally aligned fibers, which indicates the formation of perpendicular fibers. More fibers were collected with the TPU/PAA solution than with the TPU solution during electrospinning as well. Therefore, it can be concluded that increasing solution conductivity can dramatically improve the degree of fiber orientation in the electric field and improve fiber collection. This is because the high electrical field near the plate electrodes helps to attract and align the polymer solution jet, which has high conductivity.^{37,38}

In addition, the fiber diameter statistical results of unidirectionally and orthogonally aligned fibers from different solutions are listed in Table I. Overall, the orthogonal fibers showed slightly smaller fiber diameters than the aligned fibers of the same solution, although the difference was not statistically significant at $p < 0.05$. Normally a larger gap size will lead to a larger fiber length and more fiber stretch;³⁹ however, in the current case, the difference in gap size (3 cm vs. 4 cm) might not have been large enough to generate a significant difference in fiber diameter. The TPU/CNT fibers showed significant larger diameters than the TPU fibers, whereas TPU/PAA had significant smaller fiber diameters than TPU fibers. The increase of fiber diameter by CNT might be attributed to the enhancement of the TPU/CNT solution viscosity and the presence of CNT in the electrospun fiber, since higher solution viscosity and lower solution conductivity would usually induce larger fiber diameters.^{23,38} The PAA, on the contrary, was ionized in the solution and helped to reduce solution pH value and solution viscosity, which improved TPU solubility and further induced higher electrical forces that assisted in stretching and aligning the fibers.

Cell growth

The TPU/PAA unidirectionally and orthogonally aligned fibers were chosen for cell viability and cell migration studies due to their well oriented fiber structure. The electrospun random TPU/PAA fibers and TCP were tested as control groups. The results of control groups are showed in the Supporting Information Figure S4. 3T3 fibroblasts were seeded and cultured on the samples for up to 10 days. The day 3 cell culture results are shown in Figure 6(a). The fluorescence images indicate populations of mostly live cells (green color) with a few dead cells (red color) on both unidirectionally and orthogonally aligned fibers. The cells aligned along the fiber orientation direction on the aligned fibers and exhibited a stretched spindle shape [Fig. 6(a-i,iii)]. On the orthogonal fibers, the cells orientated in two directions and most cells still presented a stretched shape [Fig. 6(a-ii,iv)]. The cytoskeleton insets show parallel-aligned cells on unidirectionally aligned fibers and perpendicular-aligned cells on orthogonally aligned fibers. After 10 days of culture [Fig. 6(b)], there were significantly more live cells on both unidirectionally and orthogonally aligned fibers, with

very few dead cells observed. Based on the cytoskeleton insets and SEM images, the fibroblasts still exhibited good alignment and presented as flat spindle shapes on the fibers, which indicates good interaction and bonding between the cells and the biomaterial substrate.⁴⁰

The cell count statistical results shown in Figure 6(c) indicate significant cell proliferation from day 3 to day 10, with significantly more cells on unidirectionally aligned fibers than on orthogonally aligned and random fibers at the day 10 time point. The TCP, however, showed the highest cell count due to the surface plasma treatment. The electrospun fibers did not go through any surface treatment process. The cell viability statistical results did not show a significant difference among all groups, while the cell viability was more than 80% at day 3 and more than 90% at day 10. Therefore, these results suggest that the electrospun unidirectionally and orthogonally aligned TPU/PAA fibers have good biocompatibility with fibroblasts.

Cell migration

Cell migration behavior was further investigated on the unidirectionally and orthogonally aligned fibers. The results are shown in Figure 7 and the videos are available online. It was observed that most cells migrated along the fiber orientation direction on the unidirectionally aligned fibers [Fig. 7(a-i,ii)] and could travel among different fibers and change movement directions back and forth. On the orthogonally aligned fibers, cells mostly migrated along the fibers as well, but they could change migration direction by 90° at the orthogonal fiber intersections as shown in Figure 7(b-i,ii). The cells cultured on random fibers and on TCP [Fig. 7(c,d-i and ii)], however, migrated randomly in all directions without certain directionality. The cell orientation results at the final stage showed that the cells orientated toward the fiber alignment direction on unidirectionally aligned fibers, with about 80% of the cells were aligned with orientation angles between -10° and 10° [Fig. 7(a-iii)]. On the orthogonally aligned fibers, 30% of the cells had orientation angles between -10° and 10°, and 20% of the cells had orientation angles between 80° and 100° [Fig. 7(b-iii)]. The reason more cells oriented toward 0° might have been because the 0° direction was the last deposited layer for the orthogonally aligned fibers. The cells cultured on random fibers and TCP had a wide orientation distribution indicating an absence of cell directionality. The cell velocity [Fig. 8(a)] and cell migration distance [Fig. 8(b)] showed similar trends. Cells seeded on unidirectionally and orthogonally aligned fibers showed significant higher cell migration velocity than the cells on random fibers and TCP, and significant higher cell migration distance than cells on TCP. These results suggest that cells migrate faster and travel farther with the guidance of orientated fibers.

Therefore, the electrospun unidirectionally aligned and orthogonally aligned TPU/PAA fibers can effectively direct cell growth, migration, and orientation, and further improve cell migration velocity and distance. These preliminary findings may be useful in the investigation of cell behaviors at the tissue level, such as organogenesis or cancer metastasis. Increased cell alignment and migration have also been shown to be linked to stem cell differentiation and gene expression. For example, Bashur et al.⁴¹ found that the formation of a ligament-like tissue from mesenchymal progenitor cells was enhanced when the scaffolds

consisted of aligned submicron fibers. Chew et al.⁴² proved that aligned PCL fibers could affect gene expression of human Schwann cells and stimulate cell maturation. Moreover, the different fiber orientations (unidirectionally aligned or orthogonally aligned fibers) might be able to provide scaffolding for engineered myocardial tissues, as muscle fiber alignment is a critical component of myocardium physiology.

CONCLUSIONS

Electrospinning is a versatile method to prepare fibrous scaffolds. Parallel aligned electrospun fibers have been reported to direct cell behavior, while the effects of fibers oriented in orthogonal directions have not yet been investigated in detail. In this study, highly oriented unidirectionally and orthogonally aligned TPU/PAA fibers were electrospun using two custom built systems, with one consisting of two parallel copper plates and the other consisting of four orthogonal copper plates with alternating negative connections. CNT and PAA were added to TPU solutions to change the solution properties. The addition of CNT and PAA both increased solution conductivity, with PAA showing a more significant improvement. The TPU/CNT solution showed a higher viscosity than the TPU solution, while the TPU/PAA solution had a lower viscosity than the TPU solution. The improved conductivity led to better fiber orientation for TPU/PAA fibers and a higher viscosity produced larger fiber diameters of the TPU/CNT fibers. 3T3 fibroblasts were cultured on the TPU/PAA fibers to investigate the cellular response to different fiber orientations. The cells showed high viability and good proliferation on the fibers. The cells could migrate along the fiber orientation directions, which was in one direction on the unidirectionally aligned fibers and in two directions on the orthogonally aligned fibers. The cell migration velocity and distance were both enhanced with the guidance of orientated fibers compared to cells cultured on random fibers and TCP. Although not investigated in this study, the different cellular behaviors on unidirectionally and orthogonally aligned fibers may be influential in eliciting a variety of biological responses, such as differentiation and gene expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of the Wisconsin Institute for Discovery (WID) and the China Scholarship Council. This content is solely the responsibility of the authors and does not necessarily represent the official views of the aforementioned organizations.

Contract grant sponsor: National Nature Science Foundation of China; contract grant number: 51073061, 21174044

Contract grant sponsor: Guangdong Nature Science Foundation; contract grant number: S2013020013855, 9151064101000066

Contract grant sponsor: National Basic Research Development Program 973 in China; contract grant number: 2012CB025902

Contract grant sponsor: National Institutes of Health; contract grant number: K18HL105504 (from the National Heart, Lung, and Blood Institute)

Contract grant sponsor: Graduate School of the University of Wisconsin–Madison

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FIGURE 1.

(a) Schematic illustration of the fiber collection methods: (i) two parallel plates to collect aligned fibers, and (ii) four orthogonal plates to collect orthogonal fibers by switching the negative connection of the parallel plates periodically. (b) Photograph, (c) conductivity and pH, and (d) complex viscosity of the as-prepared TPU, TPU/CNT, and TPU/PAA solutions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 2.

(a) XPS peak fitting of the C element for TPU, TPU/CNT, and TPU/PAA electrospun mats, (b) carbon bond quantitative results from the XPS test, (c) Raman spectrum of TPU, CNT, and TPU/CNT electrospun mats. PAA could not be detected using the Raman test. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 3.

SEM images of the (a) unidirectionally aligned and (c) orthogonally aligned fibers prepared using a TPU solution at (a), (c) low magnification and (b), (d) high magnification. (e) The fiber orientation angles of unidirectionally and orthogonally aligned fibers.

FIGURE 4.

SEM images of the (a) unidirectionally aligned and (c) orthogonally aligned fibers prepared using a TPU/CNT solution at (a), (c) low magnification and (b), (d) high magnification. (e) The fiber orientation angles of unidirectionally and orthogonally aligned fibers.

FIGURE 5.

SEM images of the (a) unidirectionally aligned and (c) orthogonally aligned fibers prepared using a TPU/PAA solution at (a), (c) low magnification and (b), (d) high magnification. (e) The fiber orientation angles of unidirectionally and orthogonally aligned fibers.

FIGURE 6.

3T3 fibroblast cell culture results on TPU/PAA fibers. (a) Day 3 and (b) day 10 cell culture results: (i, ii) fluorescence images and (iii, iv) SEM images on (i, iii) aligned fibers and (ii, iv) orthogonal fibers. The insets are the cytoskeleton images of cells on corresponding electrospun fibers. (c) MTS cell count and (d) cell viability statistical results on unidirectionally and orthogonally aligned fibers at day 3 and day 10. ($p < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 7.

Cell migration results on (a) aligned fibers, (b) orthogonal fibers, (c) random fibers, and (d) TCP from day 2 to day 5. (i) The initial state images of cell migration, (ii) traces of tracked cells, and (iii) cell orientation angle distribution frequency. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 8.

(a) The cell migration velocity and (b) the Euclidean distance cells traveled on unidirectionally aligned, orthogonally aligned, and random fibers and TCP ($p < 0.05$).

TABLE I

Fiber Diameter of Unidirectionally and Orthogonally Aligned Fibers of Electrospun TPU, TPU/CNT, and TPU/PAA

	TPU	TPU/CNT	TPU/PAA
Aligned fiber	645 ± 74	753 ± 121	582 ± 66
Orthogonal fiber	623 ± 89	738 ± 138	553 ± 74

Results are showed as an average ± standard deviation. The unit of fiber diameter is nm.