Published in final edited form as:

J Mater Chem B. 2019 January 14; 7(2): 224–232. doi:10.1039/C8TB01258J.

Electrospinning of Highly Aligned Fibers for Drug Delivery Applications

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Abstract

Electrospinning is a straightforward, cost-effective, and versatile technique for fabrication of polymeric micro/nanofibers with tunable structural properties. Controlling the size, shape, and spatial orientation of the electrospun fibers is crucial for utilization in drug delivery and tissue engineering applications. In this study, for the first time, we systematically investigate the effect of processing parameters, including voltage, syringe needle gauge, angular velocity of rotating wheel, syringe-collector distance, and flow rate on the size and alignment of electrospun PLGA fibers. Optimizing these parameters enabled us to produce highly aligned and monodisperse PLGA fibers (spatial orientation > 99% and coefficient of variation < 0.5). To assess the effect of fiber alignment on the release of encapsulated drugs from these fibers, we incorporated dexamethasone, an anti-inflammatory drug, within highly-aligned and randomly-oriented fibers with comparable diameters (~0.87 μm) and compared their release profiles. *In-vitro* release studies revealed that the aligned fibers had less burst release (~10.8% in 24 hr) and more sustained release (~8.8% average rate of change for 24 days) compared to the random fibers. Finally, the degradation modes of the aligned and random fibers after 25 days incubation were characterized and compared. The findings

Conflicts of interest

There are no conflicts to declare.

of this study can be applied for the development of 3D degradable aligned fibers for controlled drug release and tissue engineering applications.

Graphical Abstract

1. Introduction

Generation of electrospun fibers with controlled size, surface morphology, and alignment is crucial for the development of electronic, photonic, and biomedical devices.¹ The electrospinning process utilizes electrohydrodynamic phenomena to draw fibers from a solution.² This technique is relatively cost-effective and simple for fabrication of ultrathin fibers from organic and organic/inorganic solutions.^{3, 4} The electrospun fibers have been widely used in electronics and photonics^{5, 6} (e.g. organic light emitting diodes⁷, photovoltaics⁸, and field effective transistors⁹), and bioengineering¹⁰ (e.g. tissue engineering¹¹, drug delivery¹², neural interfaces^{13, 14}, biosensors^{15, 16}, and wound dressing¹⁷). High molecular weight polymers, such as poly(vinyl pyrrolidone), poly(ϵ -caprolactone), poly(methyl methacrylate), and poly(ethylene oxide) have been mixed with conducting polymers such as poly(3-hexylthiophene)¹⁸ and polyaniline to generate electrospun conductive fibers for electronic devices.^{19, 20} In Biomedical applications, a wide variety of natural and synthetic polymeric fibers have been produced using the electrospinning process.^{21, 22} Among these polymers, poly(lactic-co-glycolic acid) (PLGA), a FDA-approved biodegradable polymer, has become particularly popular for drug delivery purposes^{23–25} due to its excellent biocompatibility, biodegradability, and high encapsulation

efficiency for a broad range of compounds, including small hydrophobic drugs.^{26–28} Moreover, the degradation of PLGA can be tuned by changing the lactic to glycolic acid ratio and thus, the rate of drug release can be precisely tailored.^{28–30}

The traditional electrospinning set up on a stationary substrate generates a nonwoven mat of randomly oriented fibers due to the bending instability of the highly charged jet.^{2, 31} This nonwoven fiber mat provides a three-dimensional (3D) porous structures that can support cellular attachment and growth and is thus, of interest for wound dressing and tissue engineering.^{17, 32} In contrast, uniaxially aligned fibers can be produced on a rotating drum³³, edge of a rotating wheel³⁴, or between a pair of split parallel substrates.³⁵ The control of spatial orientation of fibers can enhance their electrical and optical properties and ultimately improve the performance of electronic and photonic devices^{36, 37}, whereas the nonwoven fiber mat morphology interrupts a charge transport process by limited fiber-to-fiber contacts and scattered transport directions along unaligned fibers.

Although the electrospinning process is quite straightforward, several parameters must be carefully adjusted to achieve spinnable regime and, at the same time, control size, alignment, and morphology of fibers. These parameters can be categorized into processing parameters (including electrical field, flow rate, and syringe needle gauge), material parameters (including viscosity, ionic conductivity, molecular weight) and ambient parameters (including humidity and temperature).^{38, 39} Although the material and ambient parameters are considered as more decisive factors for spinnability and uniform shape of the fibers, processing parameters can also significantly influence the size and alignment of the produced fibers.⁴⁰ For instance, we previously demonstrated that the polymer solution concentration and flow rate could alter the shape and size of the randomly oriented fibers, respectively.^{41, 42} Zhou *et al.* reported that the needle gauge and electrical field had a drastic impact on the size of fibers. These authors found that the thinner fibers were formed as the size of needle and electrical field increased.⁴³ In another study, Zhao *et al.* showed that increasing the flow rate led to generation of thicker fibers.⁴⁴

While previous studies have reported on the effect of electrospinning parameters on size and alignment of the resulting fibers^{43, 44}, a comprehensive investigation to: (1) identify the effect of processing parameters on physical properties of fibers and (2) determine the optimized processing parameters that enhance the alignment of fibers; with identical materials parameters and ambient conditions is essential for reducing the cost of fabrication of large-area arrays of fibers and enhancement of device performance.⁴⁵ In particular, for the drug delivery applications the release profile may be affected by the orientation of the fibers.

Here we report, for the first time, a systematic study on processing parameters influencing the morphology of electrospun aligned PLGA fibers. Through optimization of these factors, we were able to generate highly aligned monodisperse fibers (with spatial orientation of >99% and coefficient of variation of <0.5%). We hypothesized that the alignment of the fibers has an impact on the release of encapsulated drug from these fibers. To test this hypothesis, dexamethasone (DEX), an anti-inflammatory drug, was incorporated within randomly oriented and highly aligned PLGA fibers with comparable size and fiber density, and the DEX release profiles were compared. One attractive feature of electrospinning is its

ability to generate fibers with nearly uniform size.⁴⁶ Moreover, this method can be utilized to create a uniform dispersion of drugs within a polymeric matrix, with high loading capacity and drug encapsulation efficiency.^{47, 48}

2. Experimental

2.1. Materials

Poly(lactide-co-glycolide) (PLGA) (75:25 8.5E) was purchased from Lakeshore Biomaterials. Dichloromethane (DCM) and chloroform were purchased from Sigma Aldrich. Chloroform (99.8%) was obtained from Acros-Organics. Organic soluble dexamethasone (DEX) was purchased from Enzo Company. Phosphate buffered saline (PBS) tablets was purchased from Fisher Scientific. N-type Si wafers insulated with SiO₂ were obtained from University Wafers company. Si wafers were coated with chromium and gold for thickness of 20 nm and 100 nm, respectively.

2.2. Electrospinning of aligned PLGA fibers

A homogeneous solution of 14 wt.% PLGA was prepared by dissolving 2.17 g PLGA in 10 ml DCM at room temperature. A typical electrospinning setup consisting of a syringe pump (Logato 100, kd Scientific, Figure 1A-a), a rotating wheel (diameter 25 cm, Figure 1A-b) connected to a motor (Caframo BDC6015), and a high voltage power supply (PS/ER40P07, Glassman High Voltage, Inc., Figure 1A-c) were used to produce aligned PLGA fibers. Electrospinning was performed at room temperature (25 °C) and humidity of 30%. PLGA fibers were collected on 5 mm × 20 mm plastic coverslip substrates, which were coated with 100 nm of gold layer (sputter coater Quorum EMS 150R S) and attached to the edge of the wheel (Figure 1A-b). The inner diameters of the syringe tip were 0.69 mm, 0.60 mm, 0.51 mm, 0.41 mm, and 0.34 mm for needle gauge numbers of 19, 20, 21, 22 and 23, respectively.

2.3. Size, alignment, and shape measurements

Optical images (Carl Zeiss Imager Z1, Germany) were used for qualitative assessment of fibers. Scanning electron micrographs (SEM) (Hitachi SU8230) were used for quantitative assessments. The diameter, alignment, and shape of electrospun fibers (n= 100) were characterized using Image J software (NIH) as previously reported.^{49, 50} The cylindrical shape of fibers was evaluated by measuring the change of diameter along the length of 150 μm (conical ratio).

2.4. Surface roughness measurement

Materials confocal microscopy (LSM800 Zeiss, Germany) and ConfoMap software (Zeiss, Germany) were used to generate 3D surface topography and measure the root mean square of surface roughness of PLGA fibers.

2.5. Compositional characterization

The infrared spectra of the samples were measured by Fourier Transform Infrared (FTIR) spectrometry (Cary670 spectrometer, Agilent Technologies) in the range of 850–3250 cm^{-1} at room temperature in reflection mode ($n=64$ scans per sample).

2.6. Physical Characterization

X-ray diffraction (XRD) pattern of samples were recorded over a wide range of Bragg angles ($2\theta=5^\circ\text{--}90^\circ$) with a scan rate of 0.03°s^{-1} using an X-ray diffractometer (Pan Analytical) with Cu $K\alpha$ radiation generated at 40 kV and 40 mA.

Differential scanning calorimetry (DSC) thermograms of the samples were obtained using a calorimeter (Mettler-Toledo, DSC1) in the temperature range of 0–400°C at the heating rate of $10^\circ\text{C}\cdot\text{min}^{-1}$ under nitrogen atmosphere.

2.7. Drug release

The release profile of DEX from aligned and randomly oriented PLGA fibers was investigated in 1X PBS solution. To prepare DEX-loaded PLGA solution, 993 mg PLGA (10 wt.%) and 75 mg DEX were dissolved in 6 ml chloroform by stirring over night at room temperature. Then, the prepared solution was electrospun (at room temperature 25 °C and 30% humidity) on rectangular shaped (1 cm $\times$ 0.5 cm) gold-coated Si wafers ($n=80$). To generate aligned and randomly oriented fibers with comparable diameter ($0.87\pm0.21\text{ }\mu\text{m}$) the processing parameters were accordingly adjusted. For aligned fibers, Si substrates were attached to the edge of rotating wheel and fibers were collected for 30 min using a syringe needle gauge 22, voltage (V)= 10 kV, flow rate (Q)= 400 $\mu\text{l}\cdot\text{hr}^{-1}$, collector distance (L)= 10 cm, and the collector angular velocity (w)= 1000 rpm.

Random fibers were electrospun on the Si substrates for 45 s using a syringe needle gauge 23 at voltage (V)= 10 kV, flow rate (Q)= 100 $\mu\text{l}\cdot\text{hr}^{-1}$, and collector distance (L)= 11 cm. The electrospinning time was selected 30 min for aligned fibers and 45 s for randomly oriented fibers in order to collect approximately the same amount of electrospun fibers. To test whether the amount of aligned and random fibers on Si substrates were comparable, DEX-loaded PLGA fibers were dissolved in chloroform and then the amount of the DEX was detected at 240 nm using a UV-Vis spectrophotometer (Molecular Devices SpectaMax) and compared. All samples were UV sterilized for 2 hr, then they were placed in plastic petri dishes (two same samples per petri dish) containing 6 ml PBS solution (1X, pH 7.4) and sealed by parafilm. Samples were then kept in incubator (Eppendorf 170S) at 37 °C for different time periods. Since DEX is not water soluble, to quantify the amount of DEX released from the fibers, an inverse approach was employed. To this end, first, the samples were removed from PBS at different time points and immersed in chloroform, and the unreleased DEX was detected and measured. The amount of DEX released from PLGA fibers was then calculated based on a calibration curve and by subtraction of the measured amount of unreleased DEX from the total amount of DEX loaded into PLGA fibers. To maintain the sink condition during the drug release measurements, the amount of DEX for drug encapsulation was selected according to the maximum solubility of DEX 12.5 $\text{mg}\cdot\text{ml}^{-1}$ in chloroform.

2.8. Statistical Analysis

Statistical analysis of data was performed by one-way ANOVA and Post-Hoc test (Tukey's test) to find significances between sample groups (Origin Pro, Northampton, MA). Data are presented as mean $\pm$ standard deviation (SD) (n=5) in Figure 8 and as mean $\pm$ standard error of mean (SEM) (n=100) in Figures 2 and 3.

3. Results and Discussion

A set of preliminary experiments was performed to determine the spinnable range of processing parameters, including voltage (V), flow rate (Q), needle-collector distance (L), syringe needle gauge (G), and angular velocity of rotating wheel (w), by which uniform fibers can be produced (summarized in Table 1). First, we found that a PLGA solution concentration in the range of 10–14 wt.% was required to obtain uniform fibers. The concentrations lower than 10 wt % produced beaded fibers while higher than 14 wt% did not generate fibers. This finding was in agreement with the PLGA concentration range previously reported by others in the literature.⁵¹ Here, we used the solution of 14 wt.% PLGA to investigate the effect of processing parameters on the size and the alignment of electrospun fibers. Figures 1B and 1C show the SEM images of aligned PLGA fibers that were produced using the processing parameters of V= 12kV, Q= 400 $\mu\text{L}\cdot\text{hr}^{-1}$, w= 1000 rpm, G= 22, and L = 14 cm. The diameter of PLGA fibers was $2.82\pm 0.7\ \mu\text{m}$ (see Figure 1S of supporting information for histogram of the size of PLGA fibers). SEM images in Figures 1B and 1C illustrate the surface morphology of highly aligned PLGA fibers (spatial orientation= $99.3\pm 0.5\%$). Figures 1D and 1E show the optical images of highly aligned ($99.3\pm 0.5\%$) and relatively less aligned ($94.7\pm 2.9\%$) PLGA fibers, respectively.

The effect of processing parameters on the diameter of aligned PLGA fibers is presented in Figure 2. As shown in Figure 2A, the diameter of fibers varied from $1.37\pm 0.02\ \mu\text{m}$ to $1.30\pm 0.02\ \mu\text{m}$, $1.33\pm 0.02\ \mu\text{m}$, and $1.31\pm 0.02\ \mu\text{m}$ as the voltage (V) increased from 6kV to 8kV, 10kV, and 12kV, respectively. Although not significant, the observed trend of decreased diameter with increasing the voltage is in agreement with the prior reports. For instance, Gu *et al.* reported that the average diameter of randomly oriented polyester fibers decreased as the applied voltage increased.⁵² According to Figure 2B, the diameter of fibers significantly decreased ($p < 0.001$ and $p < 0.01$) as the syringe needle gauge number increased from 19 to 23. The resultant fiber diameters at various angular velocities of 100, 200, 400, 600 and 1000 rpm were $1.61\pm 0.07\ \mu\text{m}$, $1.42\pm 0.03\ \mu\text{m}$, $1.35\pm 0.03\ \mu\text{m}$, $1.23\pm 0.03\ \mu\text{m}$ and $1.3\pm 0.02\ \mu\text{m}$, respectively (Figure 2C). This decrease in diameter can be attributed to viscoelastic properties of PLGA, resulting in formation of thinner fibers. There was statistically significant difference in diameter of fibers between angular velocities 100 rpm and 400 rpm, 100 rpm and 600 rpm, 100rpm and 1000rpm ($p < 0.001$), and 200 rpm and 600 rpm ($p < 0.01$). In addition, the coefficient of variation (CV) of the fiber diameters ($\text{CV} = \sigma / \langle d \rangle$, where $\langle d \rangle$ and σ denote the mean and standard deviation, respectively) decreased from 43% to 24%, 22%, 20%, and 15% when the angular velocity increased from 100 rpm to 200 rpm, 400 rpm, 600 rpm and 1000 rpm, respectively. These results suggested that fibers with significantly narrower size distributions can be produced at higher angular velocities.

The effect of needle tip-collector distance (L) on the size of fibers is shown in Figure 2D. The diameter of fibers was $1.36 \pm 0.02 \mu\text{m}$, $1.52 \pm 0.02 \mu\text{m}$, $1.47 \pm 0.03 \mu\text{m}$, $1.42 \pm 0.02 \mu\text{m}$ and $1.46 \pm 0.02 \mu\text{m}$ for the distances of 8 cm, 10 cm, 12 cm, 14 cm, and 16 cm, respectively. While in this case no trend was observed for the diameter of fibers, the size of fibers obtained at the collector distance of 8 cm was significantly less than all the other groups ($p < 0.001$ and $p < 0.01$) except collector distance 14 cm. The effect of flow rate (Q) on the size of fibers is illustrated in Figure 2E. The diameters of $1.28 \pm 0.02 \mu\text{m}$, $1.26 \pm 0.02 \mu\text{m}$, $1.3 \pm 0.03 \mu\text{m}$, and $1.26 \pm 0.02 \mu\text{m}$ were obtained using the flow rates of $250 \mu\text{L} \cdot \text{hr}^{-1}$, $300 \mu\text{L} \cdot \text{hr}^{-1}$, $350 \mu\text{L} \cdot \text{hr}^{-1}$ and $400 \mu\text{L} \cdot \text{hr}^{-1}$, respectively. There was no significant difference between the examined groups. The flow rate range of $250\text{--}400 \mu\text{L} \cdot \text{hr}^{-1}$ was selected because we did not observe formation of fibers below and above this range. In fact, when the flow rates were lower than $250 \mu\text{L} \cdot \text{hr}^{-1}$, the conical meniscus formed at the syringe tip rapidly solidified. The flow rate higher than $400 \mu\text{L} \cdot \text{hr}^{-1}$ produced larger droplets at the syringe tip, resulting in less charge density for required electrostatic repulsive forces to overcome the surface tension of the solution and form fibers.⁵³

The effect of the examined process parameters on the alignment of PLGA fibers is shown in Figure 3. As the voltage increased from 6 kV to 8 kV, 6 kV to 12 kV, 8 kV to 12 kV, and 10 kV to 12 kV, the fibers alignment significantly decreased from $99.4 \pm 0.04\%$ to $99 \pm 0.07\%$, $99.4 \pm 0.04\%$ to $98.2 \pm 0.11\%$, $99 \pm 0.07\%$ to $98.2 \pm 0.11\%$, and $99.1 \pm 0.08\%$ to $98.2 \pm 0.11\%$, respectively ($p < 0.001$) (Figure 3A). The calculated CV showed monodispersity in alignment⁵⁴ for all the groups (i.e. CV of 0.43%, 0.65%, 0.77% and 1.11%, for voltages 6 kV, 8 kV, 10 kV, and 12 kV, respectively). However, it should be noted that the CV increased about 160% when the voltage increased from 6 kV to 12 kV. This result might be attributed to attraction of fibers toward other metallic parts of the wheel, resulting in reduced alignment.

Figure 3B demonstrates the effect of needle gauge number on the alignment of fibers. As depicted, by increasing the needle gauge number, alignment (i.e. spatial orientation) of uniaxial fibers was enhanced. The alignment of fibers was increased from $97.77 \pm 1.0.14\%$ to $98.9 \pm 0.0.08\%$, $98.84 \pm 1.0.11\%$, $99.19 \pm 0.0.07\%$ and $99.35 \pm 0.0.07\%$ as the needle gauge number increased from 19 to 20, 21, 22 and 23, respectively. A statistically significant difference was found between all groups ($p < 0.001$) except between gauge numbers 20 and 21, 20 and 22, and 21 and 22. The wheel angular velocity (ω) also had a significant influence on the fiber alignment. As shown in Figure 3C, the alignment of fibers was significantly enhanced from $96.04 \pm 0.45\%$ to $97.59 \pm 0.29\%$, $98.68 \pm 1.0.12\%$, $98.27 \pm 0.17\%$ and $99.59 \pm 0.03\%$ by increasing the angular velocity from 100 rpm to 200 rpm, 400 rpm, 600 rpm and 1000 rpm, respectively ($p < 0.01$ and $p < 0.001$). However, no significant different was observed between the angular velocities 200 rpm and 400 rpm, 200 rpm and 600 rpm, and 600 rpm and 1000 rpm. The calculated CV showed narrow alignment distribution was achieved at higher angular velocity ($\text{CV} < 5\%$).^{54, 55} The CV of alignment was 3.33%, 1.95%, 1.17%, 1.7% and 0.34% for angular velocities of 100 rpm, 200 rpm, 400 rpm, 600 rpm and 1000 rpm, respectively.

The effect of needle tip-collector distance (L) on the alignment of PLGA fibers is presented in Figure 3D. The fiber alignments $97.69 \pm 0.17\%$, $97.04 \pm 0.11\%$, $98.94 \pm 0.07\%$,

98.77±0.06% and 96.9±0.12% were obtained for the collector distances 8 cm, 10 cm, 12 cm, 14 cm and 16 cm, respectively. Although there was not any specific trend, the narrowest alignment distribution was found for the collector distance of 12 cm (CV= 0.65%). There was statistically significant difference between all groups ($p<0.001$) except between the collector distances 10 cm and 16 cm. The fiber alignments 99.23±0.05%, 98.84±0.09, 99.32±0.05% and 99.2±0.07% were achieved for the flow rates 250 $\mu\text{L}\cdot\text{hr}^{-1}$, 300 $\mu\text{L}\cdot\text{hr}^{-1}$, 350 $\mu\text{L}\cdot\text{hr}^{-1}$ and 400 $\mu\text{L}\cdot\text{hr}^{-1}$, respectively (Figure 3E). While no specific trend was observed, there was statistically significant difference between the flow rates 250 $\mu\text{L}\cdot\text{hr}^{-1}$ and 300 $\mu\text{L}\cdot\text{hr}^{-1}$, and between the flow rates 300 $\mu\text{L}\cdot\text{hr}^{-1}$, 350 $\mu\text{L}\cdot\text{hr}^{-1}$ and 400 $\mu\text{L}\cdot\text{hr}^{-1}$ ($p<0.001$).

Confocal materials microscopy was utilized to reconstruct 3D height map (Figure 4A) and measure the root mean square (RMS) roughness of the PLGA fibers (Figure 4B). The color map in Figure 4A indicates the variation in size of the electrospun fibers. The diameter of fibers was calculated 2.68±0.49 μm , indicating 5% difference between the size of electrospun fiber measured by confocal materials microscopy and scanning electron microscopy. Figure 4B illustrates the oscillation of RMS roughness along the length of fiber for a single PLGA fiber. The RMS roughness of 35.75±18.41 nm and 4.99±0.11 nm was obtained for PLGA fibers and bare gold (control), respectively. To evaluate the cylindrical shape of aligned PLGA fibers, the conical ratio was calculated using Equation (1) by measuring fiber diameters D_1 and D_2 for a fiber length (L) of 150 μm (Figure 4C). The results showed that the conical ratio of the fibers was 1.09±0.21 over a length of 150 μm , indicating a good level of consistency in cylindrical shape of fibers (93%).

$$\text{Conical ratio} = D_2/D_1 \quad (\text{Equation 1})$$

To investigate the effect of spatial orientation of PLGA fibers on drug release profile, DEX was incorporated within the aligned and the randomly oriented PLGA fibers (drug to polymer ratio of 1:13.2) with comparable sizes (diameter= ~0.87 μm). We first calculated DEX encapsulation efficiency in PLGA fibers. The drug encapsulation efficiency was found to be ~85%. As anticipated, the high drug encapsulation efficiency in electrospun fibers may be attributed to solubility of both drug and polymer in organic solvent and minimum drug loss during encapsulation process.⁵⁵ The SEM images of the DEX-loaded randomly oriented and aligned fibers are shown in Figure 5A-5D. The diameter of aligned and random fibers was 0.87±0.21 μm . FTIR spectroscopy was utilized to validate the presence of DEX within PLGA fibers. Figure 5E shows the FTIR spectrum of DEX, PLGA fibers, and DEX-loaded PLGA fibers. As depicted, the characteristic absorbance peaks of DEX are positioned around 2946 cm^{-1} (C-H), 1747 cm^{-1} (C=O), 1666 cm^{-1} (C=C), 1450 cm^{-1} (C-H) bending of alkanes, 1380 cm^{-1} (C-H and O-H bending) and 1083 cm^{-1} (C-F). The FTIR spectrum of PLGA fibers indicates the absorbance peaks at 2946 cm^{-1} (C-H), 1747 cm^{-1} (C=O), 1450 cm^{-1} (C-H) bending alkanes, 1380 cm^{-1} (C-H and O-H bending). In the case of DEX-loaded PLGA fibers, the absorbance peaks of C=C (1666 cm^{-1}) and C-F (1083 cm^{-1}), which are attributed to the characteristic peaks of DEX, revealed the presence of DEX in the PLGA fibers. The characteristic absorbance wavelengths obtained for DEX, PLGA fibers and

DEX-loaded PLGA fibers are in good agreement with those previously reported in literature.
56–58

We hypothesized that the alignment of the fibers would have an effect on the release of encapsulated DEX. To assess this hypothesis, first the structural and thermal properties of both random and aligned fibers were analyzed using XRD and DSC techniques, respectively. The XRD patterns of DEX powder, random PLGA fibers, and DEX-loaded PLGA fibers (random and aligned) are shown in Figure 6. As shown in Figure 6A, the three main characteristic peaks of crystalline DEX were positioned at $2\theta = 14.2^\circ$, 15.2° , and 16.9° . The XRD spectrum of random PLGA fibers showed a broad hump in the range of 12° – 25° , which is indicative of the amorphous polymeric matrix, and represented also two crystalline peaks at $2\theta = 27.1^\circ$ and 27.8° (Figure 6B). The observation of these two crystalline peaks suggests that some ordered domains (i.e. fibrils) may exist within the amorphous matrix. These fibrillar/ordered domains were created as a result of arrangement of polymer chains induced by the elongation force applied on the polymer jet by the electric field during electrospinning.⁵⁹ As indicated in Figure 6C, by incorporation of DEX into the random PLGA fibers three small peaks, which are attributed to the main characteristic peaks of DEX, were appeared on the broad hump of PLGA spectrum. These results confirm the presence of DEX in the electrospun PLGA fibers. In the case of DEX-loaded aligned fibers (Figure 6D), the XRD pattern indicates more crystalline peaks than that of their random counterparts (Figure 6C). As shown in Figure 6D, the excess peaks are located at $2\theta = 38.5^\circ$, 43.4° , and 44.7° . The presence of excess crystalline peaks in aligned fibers is due to the formation of more ordered chain domains resulted from extra stretching forces as the fibers are collected on the rotating wheel. Thus in the case of aligned fibers, the ordered domains (i.e. fibrils) are organized along the fiber length.^{60, 61}

The DSC thermograms of DEX, PLGA random fibers, and DEX-loaded random and aligned PLGA fibers are presented in Figure 7. As indicated, the melting peak of crystalline DEX was located at 257°C (blue curve A). The DSC thermogram of PLGA random fibers (red curve B) showed a glass transition at $T_g \approx 47^\circ\text{C}$ and also a decomposition peak over 300°C . In the case of DEX-loaded PLGA fibers, the DSC thermograms of both random (black curve C) and aligned fibers (green curve D) indicated the same glass transition and decomposition peak of PLGA, but no melting peak for DEX. This implies that DEX was molecularly dispersed in the polymer matrix.⁶² In addition, these results were verified by very low intensity of the main diffraction peaks of DEX in Figures 6C and 6D, which suggests that low quantity of the incorporated DEX was in the crystalline form. Furthermore, the DSC thermograms of random and aligned fibers demonstrated a small peak at $\sim 57^\circ\text{C}$ which is likely attributed to the melting of fibrils form during electrospinning (see inset in Figure 7). As observed, the melting peak for aligned fibers (green curve D) with more ordered domains (fibrils) was more pronounced than random fibers (black curve C).

The *in-vitro* release profile of DEX from aligned and randomly oriented PLGA fibers was investigated in PBS at 37°C .³⁴ As illustrated in Figure 8A, both DEX release profiles exhibited an initial burst release during 24hr followed by a sustained release for the next 24 days. The burst release can be attributed to the tendency of accumulation of DEX molecules near the surface of fibers during electrospinning process, resulting in fast release of DEX

due to the initial surface erosion of PLGA fibers.^{12, 41} The subsequent prolonged release can be explained by bulk degradation of fibers and diffusion of drug.⁶³

There was statistically significant difference ($p < 0.05$) for initial burst release between randomly oriented fibers ($58.9 \pm 7.8\%$) and aligned fibers ($48.1 \pm 2.6\%$). The higher initial release of random fibers ($\sim 10.8\%$) is presumably due to the creation of pores between fibers of electrospun nonwoven mat that facilitate initial surface degradation of the randomly oriented fibers in comparison with aligned fibers that are packed together (SEM images in Figure 5A-5D). In contrast, over 24 days aligned fibers exhibited significantly $\sim 8.8\%$ higher rate of sustained release than their randomly oriented counterparts ($29.2 \pm 4.1\%$ for aligned fibers and $20.4 \pm 9.8\%$ for random fibers). As a consequence of higher rate of sustained release of aligned fiber, in day 25 there was no statistically significant difference for DEX release between randomly oriented fibers and aligned fibers ($77.4 \pm 3.1\%$ for aligned fibers and $79.4 \pm 5.9\%$ for random fibers). To further investigate this behavior, we characterized the surface morphology of the degraded fibers after 25 days. In contrast to randomly oriented fibers, aligned fibers exhibited a non-uniform surface morphology (as shown in SEM micrographs in Figures 8B-8E and schematics illustrated in Figures 8F and 8G). This interesting observation can be interpreted based on the different conformation of polymer chain developed during electrospinning.^{60, 61} As discussed earlier, random fibers almost hold an amorphous structure with less fibrils (Figure 8G).¹² However in the case of aligned fibers, more fibrillar domains are formed and oriented along the fiber length as a result of the excess stretching forces to the fiber stream during collection of fibers on the rotating wheel (Figure 8F).^{60, 61} The favorable hydrolysis of amorphous regions compared to the fibrillar domains in aligned fibers may contribute to anisotropic degradation of the aligned fibers (shown in Figures 8B and 8D and illustrated in Figure 8F).⁶³ On the other hand, randomly oriented fibers demonstrated an isotropic surface degradation due to their predominant amorphous structure associated with the random dispersion of some fibrillar domains (Shown in Figures 8C and 8E and illustrated in Figure 8G).⁶³ In comparison to random fibers, the higher sustained release (i.e. average rate of change) of drug from aligned fibers might be explained by the anisotropic degradation of the aligned fibers. Overall, these findings revealed the effect of fiber alignment on their drug release profile and demonstrated that aligned fibers exhibited less initial burst release and more sustained release compared to the random fibers.

4. Conclusions

In summary, we comprehensively investigated the effect of electrospinning processing parameters on the diameter and alignment of electrospun PLGA fibers. The experimental results revealed that needle gauge number, angular velocity of wheel, and collector distance have significant influence on the diameter of the produced fibers. The spatial orientation of fibers was predominantly affected by needle gauge number, voltage, and angular velocity of wheel. Highly aligned (with spatial orientation $> 99\%$) and monodisperse ($CV < 0.5\%$) fibers were produced using the optimized electrospinning parameters. Moreover, the effect of spatial orientation of fibers on their drug release behavior was assessed using DEX. The release studies revealed that aligned fibers had less burst release and more sustained release than the random fibers, presumably due to the higher porosity of random fibers and

anisotropic degradation of aligned fibers, respectively. The results of this study can be utilized for the development of highly aligned polymeric fibers for electronics and biomedical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Funding was provided by the National Institutes of Health, National Institute of Neurological Disorders and Stroke grant number R01 NS087224. We would like to thank Martin Antensteiner for his helpful comments on the manuscript and Dr. Mayerich for FTIR instrument.

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Fig 1.
(A) Schematic illustration of electrospinning setup used for production of aligned PLGA fibers: (a) syringe and syringe pump, (b) metallic rotating wheel (collector), and (c) high voltage power supply. (B-C) Representative scanning electron micrograph of highly aligned PLGA fibers. (D) Optical image of highly aligned fibers. (E) Optical image of relatively less aligned fibers.

Fig 2. Effect of electrospinning processing parameters on the diameter of aligned PLGA fibers. Bar graphs show the effects of voltage (A), needle gauge (B), angular velocity (C), needle-collector distance (D), and flow rate (E) on the fibers diameter. ** and *** indicate the significance level of $p < 0.01$ and $p < 0.001$, respectively. Data is shown in mean $\pm$ SEM ($n = 100$).

Fig 3.

Effect of processing parameters on the alignment of PLGA fibers. Bar graphs show the effect of voltage (A), needle gauge number (B), angular velocity (C), needle-collector distance (D), and flow rate (E) on fiber alignment. The symbols ** and *** indicate the significance level of $p < 0.01$ and $p < 0.001$, respectively. Data is shown in mean $\pm$ SEM (n=100).

Fig 4. (A) 3D image of aligned PLGA fibers reconstructed using material confocal microscope. (B) The graph of height map obtained from the surface of a single aligned PLGA fiber to measure RMS roughness, (C) A schematic of the conical shape of aligned fibers, and the corresponding dimensions (D_1 , D_2 , and L) used for calculation of conical ratio (Equation 1).

Fig 5. SEM images of aligned (A, C) and randomly oriented (B,D) DEX-loaded PLGA fibers at different magnifications. (E) FTIR spectrum of DEX, PLGA and DEX-loaded PLGA in the wavelength range of 850–3250 cm^{-1} .

Fig 6. XRD patterns of DEX (A), random PLGA fibers (B), DEX-loaded PLGA random fibers (C) and DEX-loaded PLGA aligned fibers (D).

Fig 7.
DSC thermograms of DEX (A, blue), random PLGA fibers (B, red), and DEX loaded random PLGA fibers (C, black) and DEX-loaded aligned PLGA fibers (D, green).

Fig 8.

(A) The release profile of DEX from PLGA aligned fibers (black squares) and randomly oriented fibers (red circles). SEM images of aligned (B, D) and random (C, E) DEX-loaded PLGA fibers after 25 days incubation in PBS. (F, G) Schematics illustrating the degradation modes of fibers. The cylindrical blocks are indicative of fibrils form during electrospinning of aligned fibers. Data is shown with $\pm$ SD (n= 5).

Table 1.

Electrospinning parameters

Voltage (V)	6, 8, 10, 12 (kV)
Flow rate (Q)	250, 300, 350, 400 ($\mu\text{L}\cdot\text{hr}^{-1}$)
Angular velocity (ω)	100, 200, 400, 1000 (rpm)
Needle gauge (G)	19, 20, 21, 22, 23
Needle tip-Collector distance (L)	8, 10, 12, 14, 16 (cm)