

## Research Article

# Influence of $\text{Fe}_3\text{O}_4$ Nanoparticles on the Preparation of Aligned PLGA Electrospun Fibers Induced by Magnetic Field

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The magnetic electrospinning (MES) method has been applied to generate aligned nanofibers. But researchers have different viewpoints on the usage of magnetic particles in the polymeric solutions. In order to investigate the effect of magnetic particles in forming the ordered fibers, the poly(lactic-co-glycolic acid) solutions with or without  $\text{Fe}_3\text{O}_4$  nanoparticles were electrospun via MES. The fibers were compared at different voltages (13.5, 15.5, 17.5, and 19.5 kV) and flow rates (0.6, 0.9, 1.2, and 1.8 mL/h). It is shown that the well-aligned fibers can be fabricated by both magnetic and nonmagnetic solutions. The doping of  $\text{Fe}_3\text{O}_4$  nanoparticles can increase the aligned fibers in some degree, especially at high applied voltage and flow rate. The diameters of fibers electrospun by MES were smaller than those by conventional electrospinning, and the diameter of fibers by MES without magnet particles was the smallest.

## 1. Introduction

Electrospinning is a simple and versatile method to generate ultrathin continuous fibers with diameters ranging from tens of nanometers to several micrometers [1–6]. These nanofibers have been explored for a wide range of applications such as tissue engineering, textiles, nanofiber reinforcement, filtration, wound dressing, drug delivery, and electrodes [7–22].

In the process of traditional electrospinning, deposited fibers are typically randomly oriented in the form of nonwoven mat because of the bend instability of charged jets of polymer solution [23–26]. The well aligned fibers can provide topographical cues to specific cells and cells cultured on aligned nanofiber scaffolds have been shown to grow in the direction of the fiber orientation. The proliferation and differentiation of many cells are influenced by the morphologies of biomaterials, such as nanoparticles, nanotubes, and also the ordered nanofibers [27–32]. Yang et al. fabricated well-aligned poly(L-lactic acid) (PLLA) fibrous scaffolds. They found neural stem cells outgrowth was parallel to the direction of aligned PLLA nanofibers [33]. Many methods have been designed to control fiber orientation to obtain aligned fibers. These methods are mostly based on using a dynamic receiver unit and/or manipulating the electrical

field. For example, a rotating mandrel as the collector was used to obtain collagen fibers aligned along the circumferential direction of the mandrel [34]. Additionally, two pieces of conductive silicon stripes separated by a gap were introduced as the collector to get aligned nanofibers suspended over the gap [35]. Moreover, using a rotating disc collector with knife edge, Theron et al. prepared highly aligned polyethylene oxide fibers [36].

Magnetic electrospinning (MES) is effective for the fabrication of well-ordered polymeric micro- and nanofibers over large areas. MES possesses several advantages over other methods. The apparatus of MES with just two magnets added to a conventional setup is comparatively simple. The resultant fibers of MES have a very good alignment and they can be easily transferred to other substrates. However, there are still some questions needed to be addressed before further usage of MES. In Jiang's research, they added 0.5 wt%  $\text{Fe}_3\text{O}_4$  nanoparticles (NPs) into the solution of poly(vinyl alcohol) and with the attendance of external magnetic field well aligned fiber arrays were obtained via electrospinning [37]. On the contrary, the resultant fibers were not aligned when they electrospun nonmagnetized solution in the magnetic field. They pointed out that adding  $\text{Fe}_3\text{O}_4$  NPs was a prerequisite for the aligned fibers. However, Liu et al. obtained

well-aligned fiber array using poly(vinyl pyrrolidone) solution without adding magnetic nanoparticles using the MES method [38].

In order to investigate the effect of magnetic particles, MES was applied to fabricate aligned fibers with or without adding  $\text{Fe}_3\text{O}_4$  NPs into poly(lactic-co-glycolic acid) (PLGA) solution. The nonmagnetic and magnetic PLGA solutions were electrospun at different applied electrical voltages and solution flow rates. The morphologies and orientation tendency of fibers with or without magnetic particles were compared in detail, so as to make sure of the influence of  $\text{Fe}_3\text{O}_4$  NPs on fiber array.

## 2. Materials and Methods

**2.1. Materials.** PLGA (D,L-LA: GA = 50 : 50,  $M_n = 5 \times 10^4$ ) was obtained from Shandong Institute of Medical Instruments (Shandong, China). Tetrahydrofuran (THF), N, N-dimethylformamide (DMF),  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ , and ammonia were all purchased from Xilong Chemical Co., Ltd (Guangdong, China).

**2.2. Fabrication of MES.** As shown in Figure 1, MES introduced an external magnetic field generated by a pair of parallel-positioned magnets. A glass syringe equipped with a stainless-steel needle (inner diameter of 0.9 mm) was used as the container of polymer solutions. The needle was connected to a high-positive-voltage power supply. A piece of aluminum foil severed as the collector with a high-negative-voltage power supply connected to it. The power supplies (Tianjin Dongwen High-Voltage Power supply Co., Ltd., China) were capable of generating direct current voltage up to  $\pm 30$  kV. Two parallel-positioned ferrite magnets with the surface magnetic field strength of 0.35 T were located on the aluminum foil and the air gap between them was maintained at 1.5 cm during the study. The distance between the tip of the needle and the alumina foil was maintained at 15 cm. The solution was extruded using a syringe pump (Cole Parmer Instrument Company, Illinois, USA) at a constant flow rate which can be easily and accurately controlled.

**2.3. Preparation and Characterization of  $\text{Fe}_3\text{O}_4$  NPs.**  $\text{Fe}_3\text{O}_4$  NPs were prepared by chemical co-precipitation as in references [39, 40].  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (0.03 mol) and  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  (0.03 mol) were dissolved in 100 mL distilled water, and then ammonia aqueous solution was poured into it with violent stirring. The black precipitate was washed several times and dried by vacuum at  $60^\circ\text{C}$  to obtain  $\text{Fe}_3\text{O}_4$  NPs. The photograph of  $\text{Fe}_3\text{O}_4$  NPs taken by transmission electron microscope (TEM, JEM-2100F, JEOL, Japan) was shown in Figure 2.

**2.4. Preparation of Electrospun Nanofibers.** The mixture of THF and DMF (V : V, 3 : 1) was used as the solvent for electrospinning. Two kinds of solutions were prepared to investigate the influence of magnetic particles. For the preparation of polymer solution without magnetic NPs, PLGA with the concentration of 24 w/v% was dissolved under gentle stirring for 2 hours to obtain a homogeneous and stable solution.

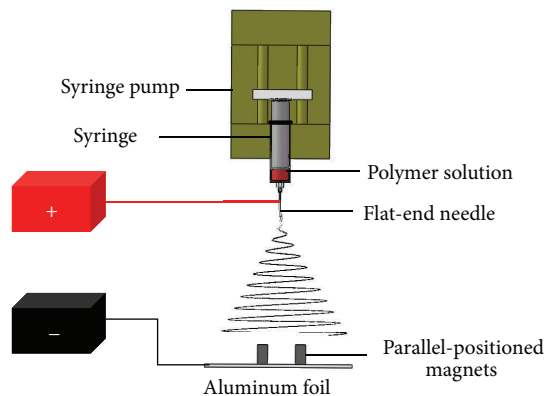


FIGURE 1: Schematic illustration of the setup used in the MES method for preparing aligned nanofibers. Two bar magnets ( $5 \times 5 \times 1$  cm) were parallel-positioned and the distance between them was maintained 1.5 cm.

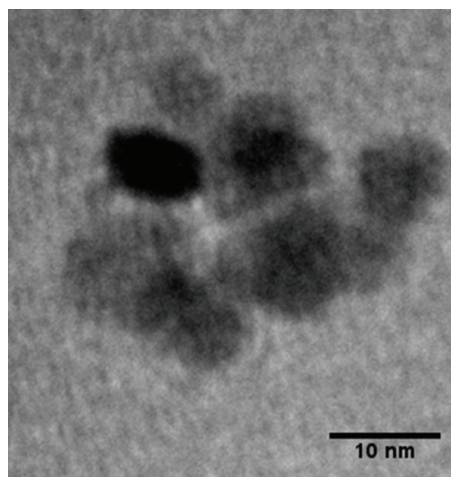


FIGURE 2: TEM photograph of the  $\text{Fe}_3\text{O}_4$  nanoparticles prepared by chemical coprecipitation.

For solution with magnetic NPs, 1 wt%  $\text{Fe}_3\text{O}_4$  magnetic NPs were dispersed into the forementioned solution without magnetic NPs for 24 hours using an ultrasonic cleaner (Tianjin Automatic Science Instrument Co., Ltd., China).

At the voltage of 15 kV and the flow rate of 0.5 mL/h, solution without magnetic NPs was electrospun through conventional electrospinning (CES). To study the effect of the electrospinning parameters, solutions were electrospun at different voltages and flow rates via MES method for 30 s. At the flow rate of 0.5 mL/h, the solutions with or without  $\text{Fe}_3\text{O}_4$  were electrospun at 13.5, 15.5, 17.5, and 19.5 kV, respectively. At the voltage of 15 kV, the solutions were electrospun at 0.6, 0.9, 1.2, and 1.8 mL/h, respectively. To compare the diameter of fibers prepared by CES and MES, the electrospun fibers with or without  $\text{Fe}_3\text{O}_4$  were produced at voltage of 15 kV and flow rate of 0.5 mL/h. The as-spun fibers were transferred to glass slides and dried for 24 hours in air before further investigation.

**2.5. Morphologies of Electrospun Nanofibers.** The morphologies of electrospun nanofibers were investigated by scanning

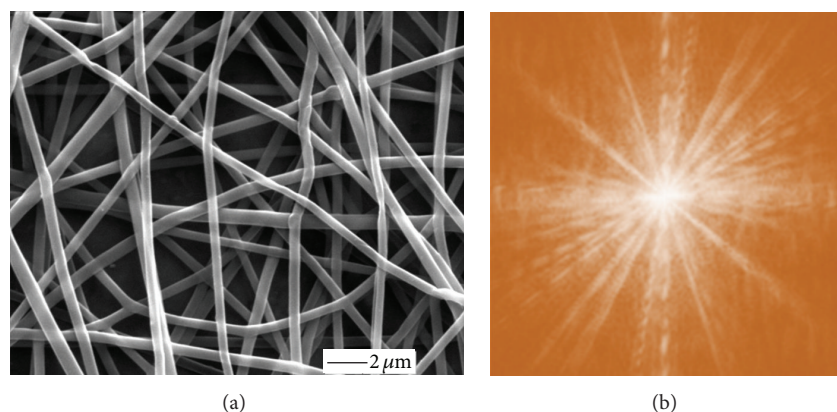


FIGURE 3: (a) SEM photographs of the nanofibers electrospun by conventional electrospinning at 24 w/v% poly(lactic-co-glycolic acid) solution, voltage of 15 kV, and flow rate of 0.5 mL/h for 30 s; (b) FFT pattern of the SEM image indicating the randomly distributed fibers.

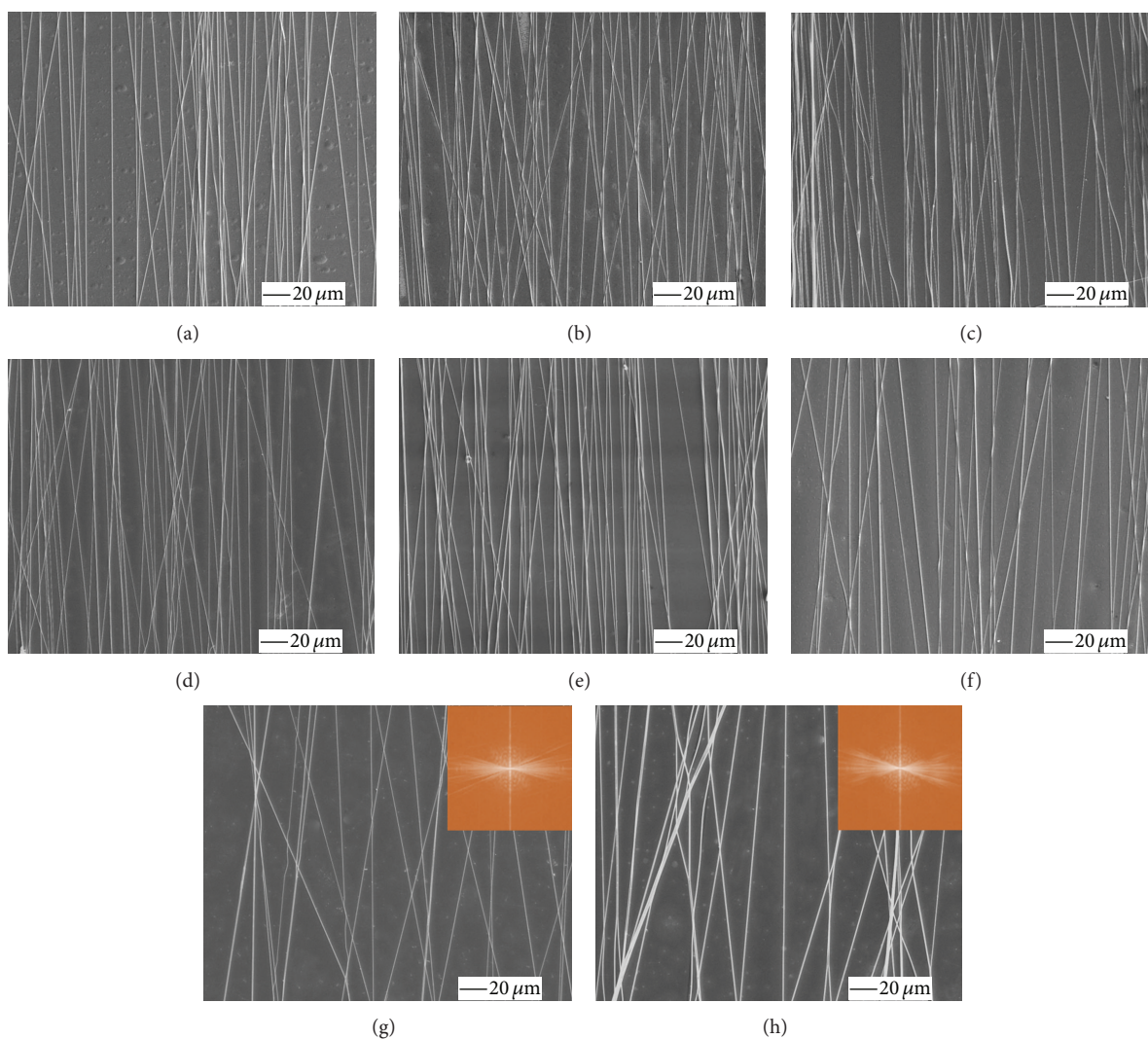


FIGURE 4: SEM photographs of aligned nanofibers fabricated via magnetic electrospinning (MES) with ((a), (c), (e), and (g)) and without  $\text{Fe}_3\text{O}_4$  nanoparticles ((b), (d), (f), and (h)) at the flow rate of 0.5 mL/h and the voltage of ((a), (b)) 13.5 kV; ((c), (d)) 15.5 kV; ((e), (f)) 17.5 kV; ((g), (h)) 19.5 kV. FFT was used to analyze the alignment of fibers in (g) and (h), respectively, as shown in the inserts.

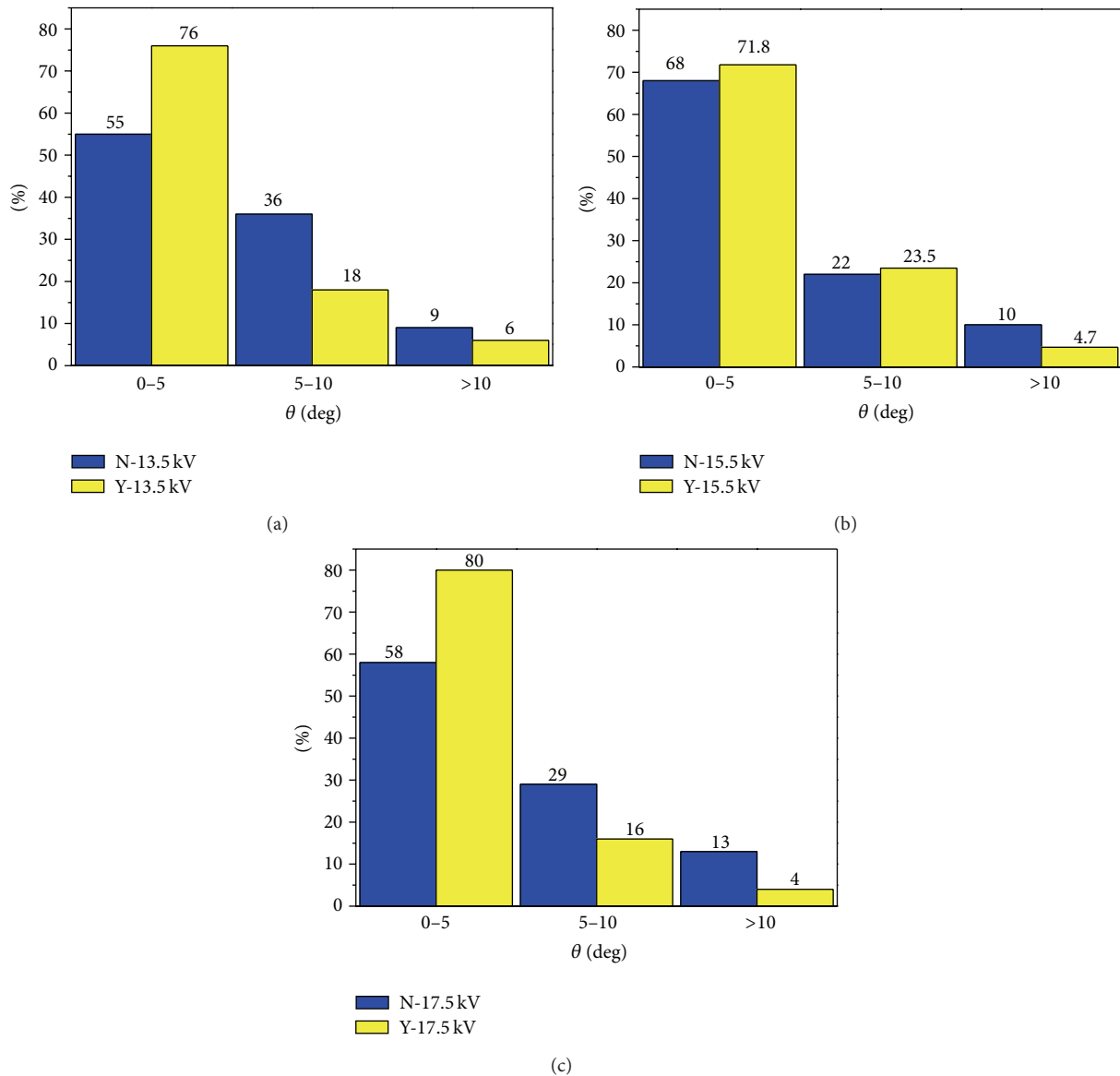


FIGURE 5: Distributions of the angles ( $\theta$ ) between the long axis of the fibers and the direction perpendicular to magnets at the flow rate of 0.5 mL/h and the voltages of (a) 13.5 kV; (b) 15.5 kV; (c) 17.5 kV. Y and N indicated the conditions with or without magnetic nanoparticles, respectively.

electron microscopy (SEM) (CS3400, CamScan, UK) after coating the nanofibers with gold.

**2.6. Analysis of Aligned Nanofibers.** The alignment of nanofibers was quantitatively assessed by the angles ( $\theta$ ) between the long axes of the fibers and the direction parallel to the vectors of the magnetic field [37]. Image J processing software was used to measure the direction of the long axes of more than 100 fibers in the SEM photographs at each condition. To analyze qualitatively the degree of fiber alignment, Fourier fast transfer (FFT) analysis was performed by utilizing the FFT function of the Image J [41]. The diameters of fibers were also measured over more than 50 fibers by Image J.

**2.7. Statistic Analysis.** All data are expressed as mean  $\pm$  standard deviation and were analyzed of variance ( $t$ -test). Significance was assigned as \* $P < 0.05$  and \*\* $P < 0.01$ .

### 3. Results and Discussions

As shown in Figure 2, the average diameter of  $\text{Fe}_3\text{O}_4$  particles measured by Image J was about 10 nm. The  $\text{Fe}_3\text{O}_4$  NPs were added in PLGA solution for MES electrospinning. In this study, CES and MES with or without magnetic NPs were used to fabricate electrospun nanofibers.

SEM image of a nonwoven mat of randomly oriented PLGA fibers by CES was shown in Figure 3(a). Figure 3(b) was the corresponding FFT pattern of Figure 3(a). The radially symmetrical silhouette (labeled with white color) in FFT

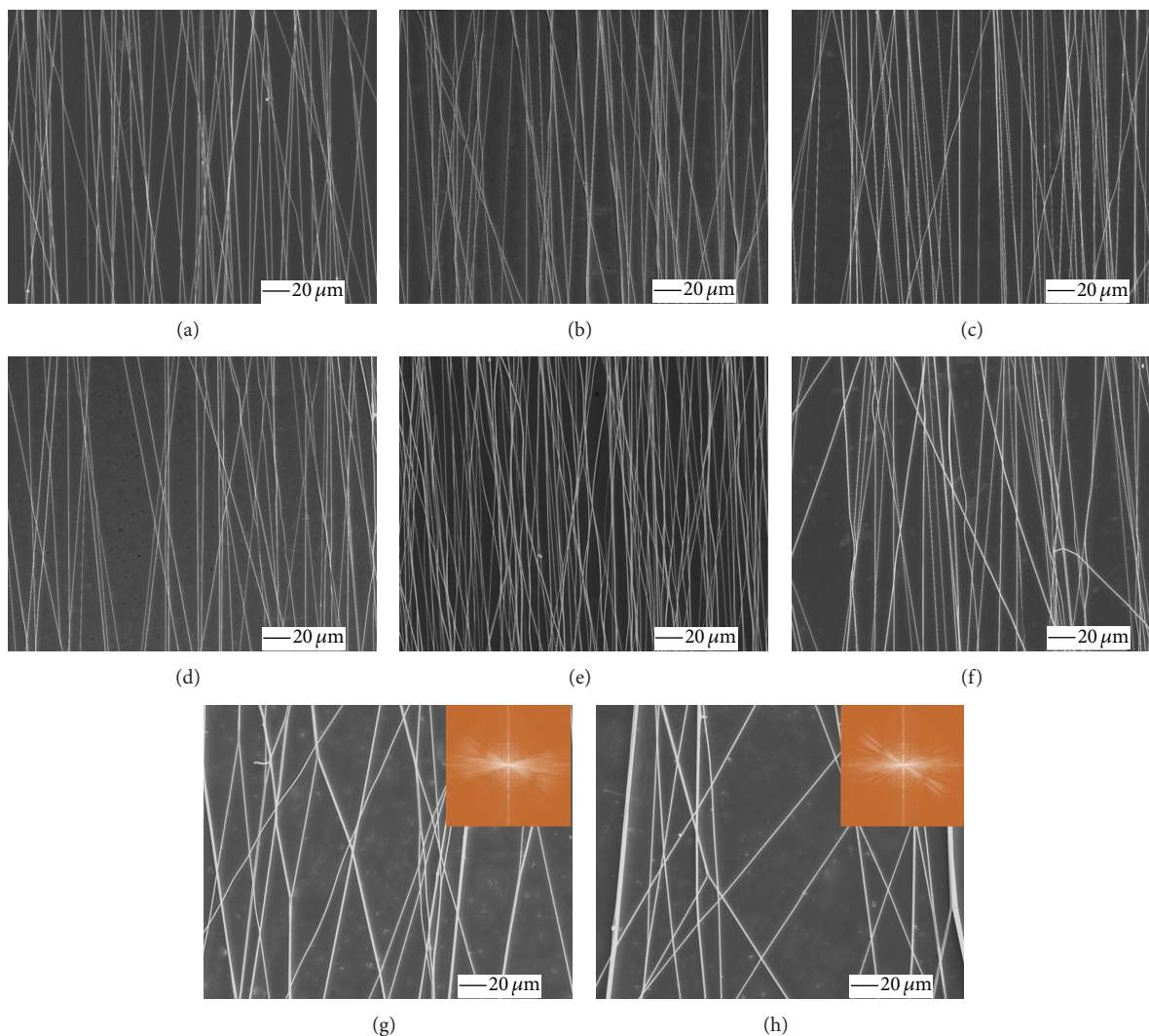


FIGURE 6: SEM photographs of aligned nanofibers fabricated via MES with ((a), (c), (e), and (g)) and without  $\text{Fe}_3\text{O}_4$  nanoparticles ((b), (d), (f) and (h)) at the voltage of 15 kV and the flow rate of (a), (b) (0.6 mL/h); (c), (d) (0.9 mL/h); (e), (f) (1.2 mL/h); (g), (h) (1.8 mL/h). The inserts in (g) and (h) were the FFT patterns of (g) and (h) respectively.

pattern is in agreement with the structure lacking directional order.

The SEM photographs of aligned fibers electrospun with or without magnetic NPs at different voltages were shown in Figure 4. At the voltages of 13.5, 15.5, and 17.5 kV, the well-aligned PLGA fibrous arrays were successfully fabricated by MES. The uniaxial aligned fibers by MES with  $\text{Fe}_3\text{O}_4$  particles were similar to those by MES without magnetic particles. It is indicated that the  $\text{Fe}_3\text{O}_4$  NPs were not the key factor to produce ordered fibers. At 19.5 kV, the alignment and uniformity of fibers with or without  $\text{Fe}_3\text{O}_4$  NPs decreased greatly. The FFT pattern inserted in Figure 4(g) showed a more compact distribution than that in Figure 4(h), which demonstrated that the alignment of fibers with  $\text{Fe}_3\text{O}_4$  NPs at high voltage was higher compared with those without magnetic particles.

At various applied voltages of 13.5, 15.5, and 17.5 kV, the orientation distributions of angles ( $\theta$ ) between the long axis of the fibers and the direction perpendicular to the magnets

were shown in Figure 5. As a whole, no obvious difference was found between the fibers by MES with or without magnetic NPs. From the analysis on alignment, there were more than 87% of fibers with or without  $\text{Fe}_3\text{O}_4$  all within  $10^\circ$  of the desired direction. The percentages of fibers aligned within  $5^\circ$  of the expected direction were 55%, 68%, and 58% for MES without magnetic NPs and 76%, 71.8%, and 80% for those with magnetic NPs. There were more fibers with magnet NPs distributed within  $5^\circ$  of the expected direction. It is shown that the magnetic NPs might increase the alignment of fibers and more fibers oriented along the direction of magnet field.

Figure 6 was the SEM photographs of fibers electrospun with or without magnetic NPs at different solution flow rates. From the SEM photographs, it could be found that the well-ordered PLGA nanofibers were also produced at the flow rates from 0.6 mL/h to 1.2 mL/h. Without adding the magnet particles, the high alignment of fibers can still be obtained. However, at the high feeding rate of 1.8 mL/h, the order

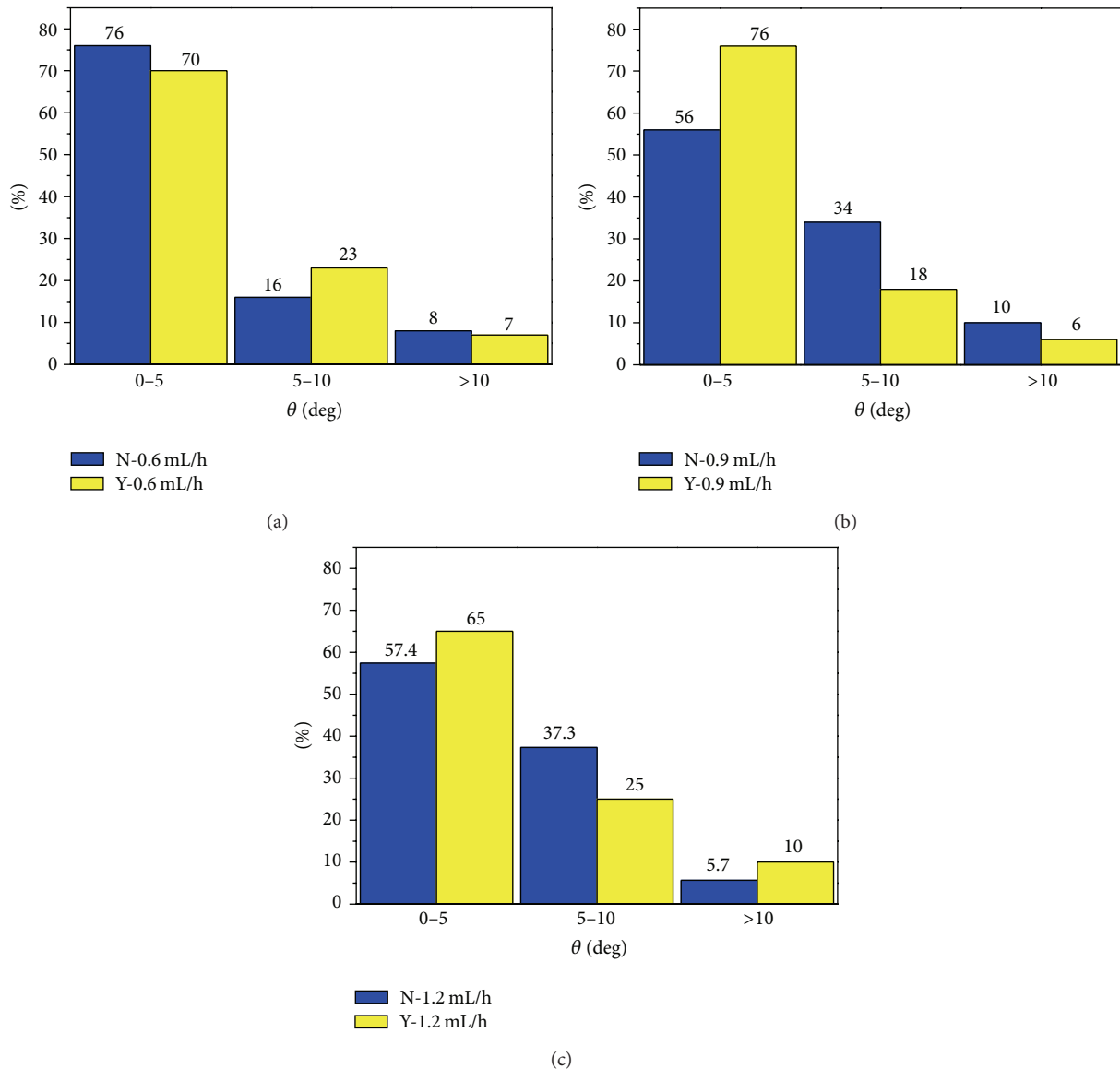


FIGURE 7: Distributions of the angles ( $\theta$ ) between the long axis of the fibers and the direction perpendicular to magnets at voltage of 15 kV and flow rate of (a) 0.6 mL/h; (b) 0.9 mL/h; (c) 1.2 mL/h. Y and N indicated the conditions with or without magnetic nanoparticles, respectively.

orientation of fibers was obviously decreased, especially for those fibers by MES without  $\text{Fe}_3\text{O}_4$  NPs. With the increasing flow rate, the alignment of fibers electrospun by MES without magnetic NPs decreased apparently, but for those electrospun with magnetic NPs, the decreasing trend of fiber alignment was smaller. The FFT pattern inserted in Figure 6(h) showed a more scattered distribution than that in Figure 6(g), which indicated that the alignment of fibers without  $\text{Fe}_3\text{O}_4$  NPs at high flow rate was lower compared with those embedding with magnetic particles.

At different flow rates of 0.6, 0.9, and 1.2 mL/h, the orientation distributions of angles ( $\theta$ ) of fibers were shown in Figure 7. Overall, there was no significant difference in the alignment between fibers by MES with or without magnetic NPs. More than 90% of fibers with or without  $\text{Fe}_3\text{O}_4$  were within  $10^\circ$  of the desired direction. The percentages

of fibers aligned within  $5^\circ$  of the expected direction were 76%, 56%, and 57.4% for MES without magnetic NPs, which presented a decreasing trend, and 70%, 76%, and 65% for those with magnetic NPs which did not show apparent decrease. More fibers with magnetic NPs distributed within  $5^\circ$  of the expected direction, except at 0.6 mL/h. At lower flow rate, the alignment of fibers electrospun by MES with or without magnetic NPs seemed to be similar. As the flow rate increased, the alignment of fibers electrospun by MES with magnetic NPs was higher than that without magnetic NPs. At high flow rate of 1.8 mL/h, although the ordered orientation decreased for both fibers, the  $\text{Fe}_3\text{O}_4$  NPs seemed to increase the alignment of PLGA fibers.

The diameter of fibers fabricated by CES and MES is analyzed by Image J, as shown in Figure 8. The diameters of fibers electrospun by CES and MES with or without magnetic

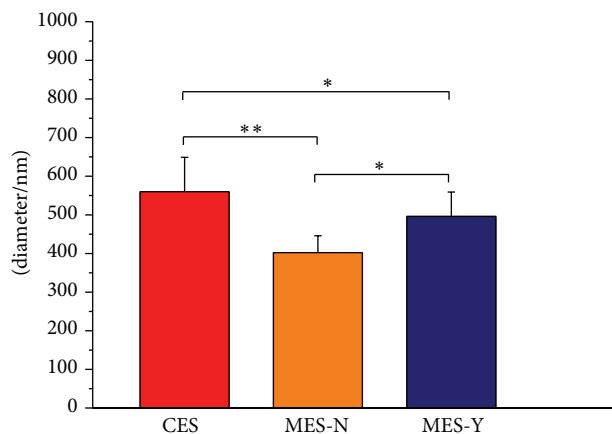


FIGURE 8: The average diameter of the electrospun fibers at the concentration of 24 w/v%, voltage of 15 kV, flow rate of 0.5 mL/h and the distance of 15 cm respectively. CES: conventional electrospinning; MES-Y and MES-N: magnetic electrospinning with or without magnetic particles. Significance was assigned as \* $P < 0.05$ , \*\* $P < 0.01$ .

NPs, at the concentration of 24 w/v%, voltage of 15 kV, flow rate of 0.5 mL/h, and the distance of 15 cm, were  $560 \pm 89$ ,  $496 \pm 63$ , and  $402 \pm 44$  nm, respectively. The results showed that MES could decrease the diameters of fibers compared to CES and fibers electrospun by MES without magnetic NPs had a smaller diameter than those with magnetic NPs.

In this work, randomly oriented nanofibers were obtained by using CES. While using polymer solution with or without magnetic NPs, well-aligned nanofibers were fabricated successfully by MES at certain voltages and flow rates, which was different from Jiang's works [37]. The results indicated that MES was a favorable method for the formation of aligned fibers and the formation of well-aligned fiber array did not depend on the magnetic NPs embedding in the solution. The formation of aligned fibers by MES might be the attendance of the external magnet field. By using CES, the spinning jet became instable after it leaves the needle tip. In the processing of MES, the charged polymer solution jet was subject to magnet field generated by the magnets and the bending instability of the polymer solution jets can be inhibited by magnetic field [42, 43]. When jets suspended over the gap between the magnets, aligned fibers were obtained. Additionally, the magnetic NPs in the fibers could also interact with the magnet to slightly adjust the orientation of the fibers and to direct more fibers along with the direction of the magnetic field lines. So for the fibers fabricated by MES with the magnetic NPs, more fibers were distributed within  $5^\circ$  to the peculiar direction of the magnet. And at high applied voltage and flow rate, more ordered nanofibers were got under MES with magnetic particles.

The decreases in fiber diameter electrospun by MES might result from an additional Lorenz force generated by the magnetic field. During MES process, the Lorenz force facilitates the charged polymeric chains to align with each fiber, which can result in the reduction of fiber diameters [38]. The reason of the increase in fiber diameter of MES

with magnetic NPs might be that magnetic NPs increased the viscosity of electrospinning solution.

#### 4. Conclusions

In this work the influence of the magnetic NPs over the alignment of the fibers at different applied voltages and flow rates during MES was demonstrated. According to the results, well-aligned PLGA fibers were successfully obtained with or without magnetic NPs. But for NPs containing fibers, more fibers were oriented within  $5^\circ$  of the peculiar direction of the magnets. At high applied voltage and flow rate,  $\text{Fe}_3\text{O}_4$  NPs can improve the alignment of fibers compared to those by MES without  $\text{Fe}_3\text{O}_4$ . The results indicated that magnetic NPs were not the critical parameter for the formation of the aligned fibers during MES, but they might be capable of slightly adjusting the direction of the as-spun fibers. MES method could also affect the diameter of the nanofibers. In comparison with the fibers fabricated by CES, the diameter of fibers by MES was smaller and the diameter of fibers without magnetic NPs was the smallest. MES is an effective method for the fabrication of well-aligned polymeric micro- and nanofibers over large areas. The mechanism of MES needs further research.

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