Conjugate Electrospinning of Continuous Nanofiber Yarn of Poly(L-lactide)/Nanotricalcium Phosphate Nanocomposite

Xinsong Li,[1] Chen Yao,[1] Fuqian Sun,[1] Tangying Song,[1] Yunhui Li,[2] Yuepu Pu[2]

[1]*Biomaterials and Drug Delivery Laboratories, College of Chemistry and Chemical Engineering, Southeast University, Nanjing 210018, People's Republic of China*
[2]*School of Public Health, Southeast University, Nanjing 210018, People's Republic of China*

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ABSTRACT: The continuous nanofiber yarns of poly(L-lactide) (PLLA)/nano-β-tricalcium phosphate (n-TCP) composite are prepared from oppositely charged electrospun nanofibers by conjugate electrospinning with coupled spinnerets. The morphology and mechanical properties of PLLA/n-TCP nanofiber yarns are characterized by scanning electron microscope, transmission electron microscope, and electronic fiber strength tester. The results show that PLLA/n-TCP nanofibers are aligned well along the longitudinal axis of the yarn, and the concentration of PLLA plays a significant role on the diameter of the nanofibers. The thicker yarn of PLLA/n-TCP composite with the weight ratio of 10/1 has been produced by multiple conjugate electrospinning using three pairs of spinnerets, and the yarn has tensile strength of 0.31cN/dtex. A preliminary study of cell biocompatibility suggests that PLLA/n-TCP nanofiber yarns may be useable scaffold materials. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 3756–3764, 2008

Key words: conjugate electrospinning; fibers; yarn; nanocomposite; strength

INTRODUCTION

Tissue engineering devices are commonly a matrix or scaffold onto which the cells are seeded. These matrices are three-dimensional (3-D) interconnected porous networks with large void volumes and high surface-to-volume ratios that allows for nutrient supply/transport while providing adequate space for cell migration and attachment within the structure. Several techniques have been utilized to construct 3-D interconnected porous matrices including fiber-bonding, solvent-casting, particle-leaching, phase separation, emulsion freeze-drying, gas-foaming, and 3D-printing technique.[1]

Recently, electrospinning has attracted more attention, since electrospun nanofibers were identified as viable candidates for tissue engineering constructs. Electrospinning is a process that uses an electric field to control the formation and deposition of polymer nanofibers. Electrospun fibers with diameters in the range of several micrometers to nanometers have a high surface area-to-volume ratio and morphology similar to natural tissues, making them excellent candidates for use in fabrication of cell-growth scaffolds, vascular grafts, wound dressings, and drug delivery.[2] The large surface area-to-volume ratio allows cellular migration and proliferation in tissue engineering scaffolds. In the past few years, a wide variety of both biologically derived and synthetic biodegradable materials have been electrospun to produce fibers.[3] However, the electrospun nanofibers are often collected as randomly oriented structures in the form of nonwoven mats, such as nanofiber mats of poly(L-lactide)/tricalcium phosphate nanocomposite.[4] Therefore, aligned nanofiber yarns of biocompatible polymers are needed to fabricate 3-D scaffold by knitting or bonding for tissue engineering.

Mechanical and electrostatic means have been explored to improve alignment of electrospun nanofibers. Fennessey and Farris have shown that electrospun fibers can be aligned more or less parallel to each other when a drum rotating at high speed is used as the collector.[5] Zussman et al. have demonstrated the use of a wheel-like bobbin as the collector to position and align individual polymer nanofibers into parallel arrays.[6] More recently, Li et al. prepared uniaxially aligned nanofibers over large areas by introducing a gap into the conventional collector.[7] Ko et al. prepared continuous carbon nanotube-filled nanofiber yarns.[8] Another method developed by Smit et al. is to deposit nanofibers into water to eliminate

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Correspondence to: X. Li (lixs@seu.edu.cn).
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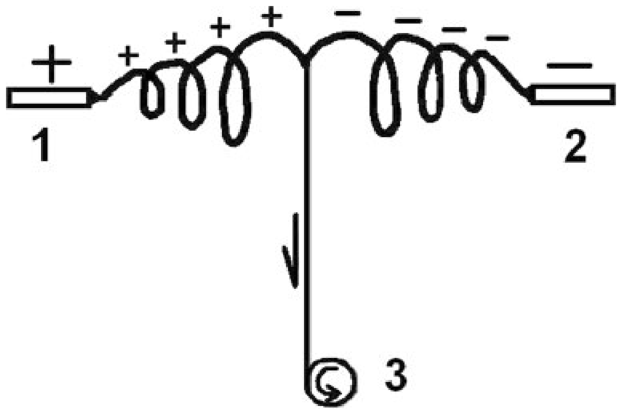

Figure 1 Schematic conjugate electrospinning and formation of nanofiber yarn using one pair of spinnerets; 1 and 2 are two opposite spinnerets connected to solution reservoirs, which are subjected to positive and negative high electrical voltage supplies, respectively; 3 is rotating receiver.

the charges of the charged nanofibers, which are collected together, and yarns are drawn out.[9] Recently, we developed a versatile method of conjugate electrospinning using spinnerets in pairs connected with two high electrical voltages of opposite polarities, respectively, which can fabricate continuous yarns from oppositely charged electrospun nanofibers.[10–13]

In present work, we investigate conjugate electrospinning of poly(L-lactide) (PLLA)/nano-β-tricalcium phosphate (n-TCP) composite to produce continuous nanofiber yarns and further fabricate 3-D scaffold for tissue engineering in the future.

EXPERIMENTAL

Materials

Poly(L-lactic acid) (PLLA) with the average viscous molecular weight of 210,000 was synthesized from L-lactide using tin 2-ethylhexanoate as catalyst.[14] β-Tricalcium phosphate (β-TCP) was synthesized according to the method in literature,[15] and nano-β-tricalcium phosphate (n-TCP) with diameter about 100 nm was obtained by grinding β-TCP in planet mill for 100 h.

Preparation of PLLA/n-TCP composite solution

PLLA was dissolved in the mixture of acetone and dimethyl formamide (DMF) with volume ratio 1/1, followed by addition of n-TCP. Three composite solutions of PLLA/n-TCP with weight ratios of 20/1, 10/1, and 5/1 were obtained, while the PLLA concentrations are 2.5, 5, and 7.5% (w/v), respectively.

Conjugate electrospinning

The apparatus used in conjugate electrospinning is shown in Figure 1.[12] It consists of two high voltage power supplies with opposite polarity, two spinnerets [Fig. 1(1, 2)], and a receiver drum [Fig. 1(3)]. Two programmable pumps were used to control the delivery rate of PLLA/n-TCP composite solution. Two power supplies are linked with two spinnerets, respectively. The two spinnerets are arranged in opposite position and same horizontal line. The distance between the two spinnerets was designed to be adjustable.

The PLLA/n-TCP composite solution was separately delivered by syringes to two spinnerets. Two high voltages with opposite polarity were applied to the two spinnerets, respectively. The syringe pump was set to deliver the solution at the rate of 5 mL/h, and the applied voltage was fixed at ±10 kV. The receiver is a rotating drum controlled by a stepping motor. The vertical distance between spinneret and receiver is about 40 cm. Under the action of electrical field, composite suspension jets on the tips of the two spinnerets were stretched out, resulting in the formation of charged nanofibers. The fibers from the two oppositely charged electrospinning spinnerets were collected and stretched by the drum receiver with a speed 45 m/min, resulting in continuous nanofiber yarns. The nanofiber yarns were dried under vacuum at room temperature.

Multiple conjugate electrospinning

Schematic setup of multiple conjugate electrospinning is shown in Figure 2. Three pairs of spinnerets arranged oppositely in two columns were used. Two mixtures of PLLA/ n-TCP composite solution are

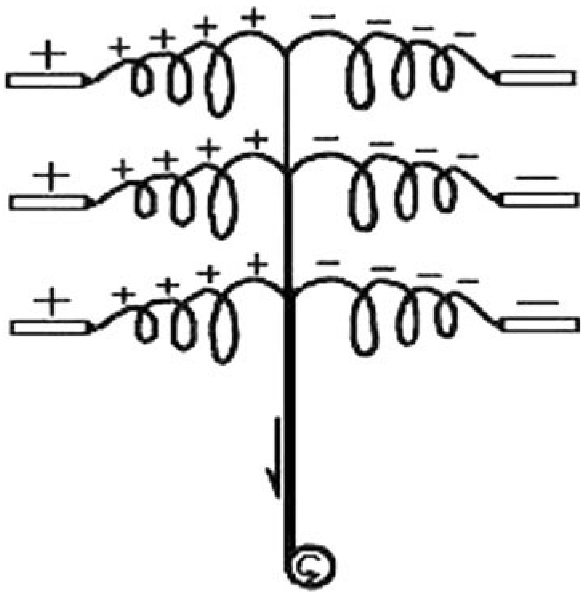

Figure 2 Schematic multiple conjugate electrospinning and formation of nanofiber yarn using three pairs of spinnerets.

delivered to two columns of spinnerets at the flow rate of 15 mL/h for each column. Then two high electrical voltages of ±15 kV with opposite polarities are applied to two column spinnerets, respectively. With similar procedure to the previous description, thicker continuous nanofiber yarns of PLLA/n-TCP composite were drawn out with a speed of 45 m/min.

Characterization of nanofiber yarns

The morphology of the nanofiber yarns was characterized by scanning electron microscope (JSM-6360LV SEM, JOEL Oxford) after gold-coating. The dispersion of n-TCP in the nanofiber was observed by transmission electron microscope (TEM, JEM2000EX, JEOL).

The mechanical properties of nanofiber yarns of PLLA/n-TCP composite were measured by electronic fiber strength tester (YG001A, Taichang Textile Instrument Factory). The continuous nanofiber yarns were electrospun from PLLA/n-TCP solution with PLLA concentration of 5% (w/v) and PLLA/n-TCP weight ratio of 10/1. Multiple conjugate electrospinning conditions: three pairs of spinnerets, power supplies of ±15 kV, distance between two spinnerets of 30 cm.

Cell compatibility of nanofiber yarns of PLLA/n-TCP composite

Cell isolation and culture

Newborn rats were killed by cervical dislocation and sprayed with 70% ethanol for sterilization. Skin from the backs of animals was dissected and stretched on the surface of a sterile petri dish after removal of most of the subcutaneous fat, and then kept at 4°C for over 40 min to obtain a good attachment of the skin to the dish. One-tenth percent of trypsin was added to the dish and kept at 4°C for cell separation. After 16 h, the enzyme solution was removed, and the skin was washed with EBSS (Earle's Balanced Salts Solution), and then to stop the enzyme reaction. After the dermis and epidermis were separated, the dermis was again digested with trypsin solution, at 37°C for 30 min; then fibroblasts cell suspension was collected, and were cultured in high-glucose DMEM (Dulbecco's modified minimum essential medium, Gibco, USA).

In vitro proliferation tests

Fibroblasts with the densities of 1.5×10^4 were seeded on nanofiber yarns assembled into cell wells and then incubated in a moisturized CO_2 incubator at 35°C, under 5% CO_2. On day 5, the materials were rinsed twice with a sterile PBS solution to remove dead cells. The 100 μL MTT [3-(4,5-dimethlthiazol-2-yl)-2,5-diphenyletrazoliem] (Sigma; USA) solution was added to each well. After 3-h incubation at 35°C, DMSO (dimethyl sulfoxide) of 200 μL was added to dissolve the formazan crystals. The dissolvable solution became homogeneous in about 15 min by the shaker.

Then an MTT assay was performed to quantify the cell viability. All experiments were repeated five times, and results are expressed as mean ± standard deviation of mean. Significance was assessed at the $P < 0.05$ level of confidence.

RESULTS AND DISCUSSION

Conjugate electrospinning of PLLA/n-TCP composites

Conjugate electrospinning of a series of PLLA/n-TCP composites were carried out using the setup as shown in Figure 1. After two high electrical voltage of 10 kV with opposite polarities are applied respectively to the two spinnerets, an electric field is formed between the two spinnerets. Under the action of the electrical field, polymer solution jets on the tips of the spinnerets are stretched out and do whipping movements, resulting in the formation of charged nanofibers. The fibers from the two oppositely charged electrospinning spinnerets carry opposite charges, which attract each other to form PLLA/n-TCP composite nanofibers. Then, the nanofibers holding together carrying opposite charges are drawn out and stretched by a drum with a speed of 45 m/min, resulting in continuous nanofiber yarn. Majority of nanofibers in the yarns are oriented along longitudinal axis which form a unique aligned topology. To explore variables that influence the structure and morphology of nanofiber yarns, concentration of polymer and distance between two spinnerets have been examined. Their relationships with the yarns microstructure are summarized below.

Concentration effect

SEM images of PLLA/n-TCP composite continuous nanofiber yarns are shown in Figure 3. It is observed that the concentration of PLLA have a significant impact on the morphology of nanofiber yarns. As the concentration of PLLA increased from 2.5% to 7.5%, the diameters of the nanofibers became bigger as revealed in Figures 3 and 4. This finding is comparable and consistent with the past research claiming that polymer concentration is the most dominant

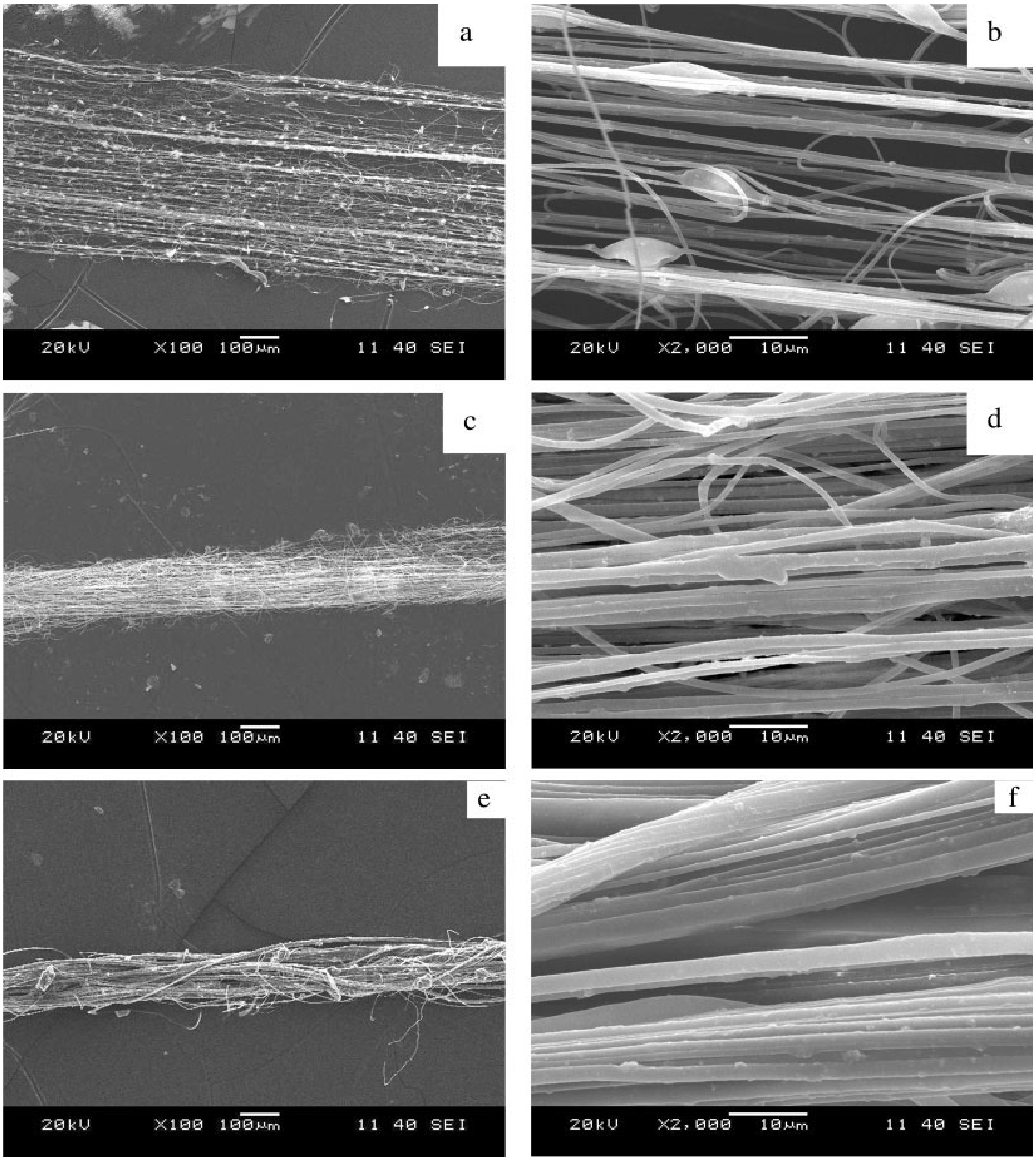

Figure 3 SEM images of PLLA/n-TCP composite continuous nanofiber yarns electrospun from PLLA/n-TCP solution with PLLA concentration of (a, b) 2.5% (w/v), (c, d) 5%, and (e, f) 7.5%, with PLLA/n-TCP weight ratio of 10/1. Conjugate electrospinning condition: power supplies, ±10 kV; distance between two spinnerets, 30 cm. Magnification: (a, c, e) 100; (b, d, f) 2000.

parameter in general electrospinning using one high voltage power supply.[16]

In the conjugate electrospinning, beaded nanofibers are obtained when the concentration of PLLA is 2.5%. The average diameter of the nanofibers is about 600 nm. The beaded nanofibers hold together to form very loose yarn as shown in Figure 3(a,b). At concentrations of PLLA higher than 5%, tightly

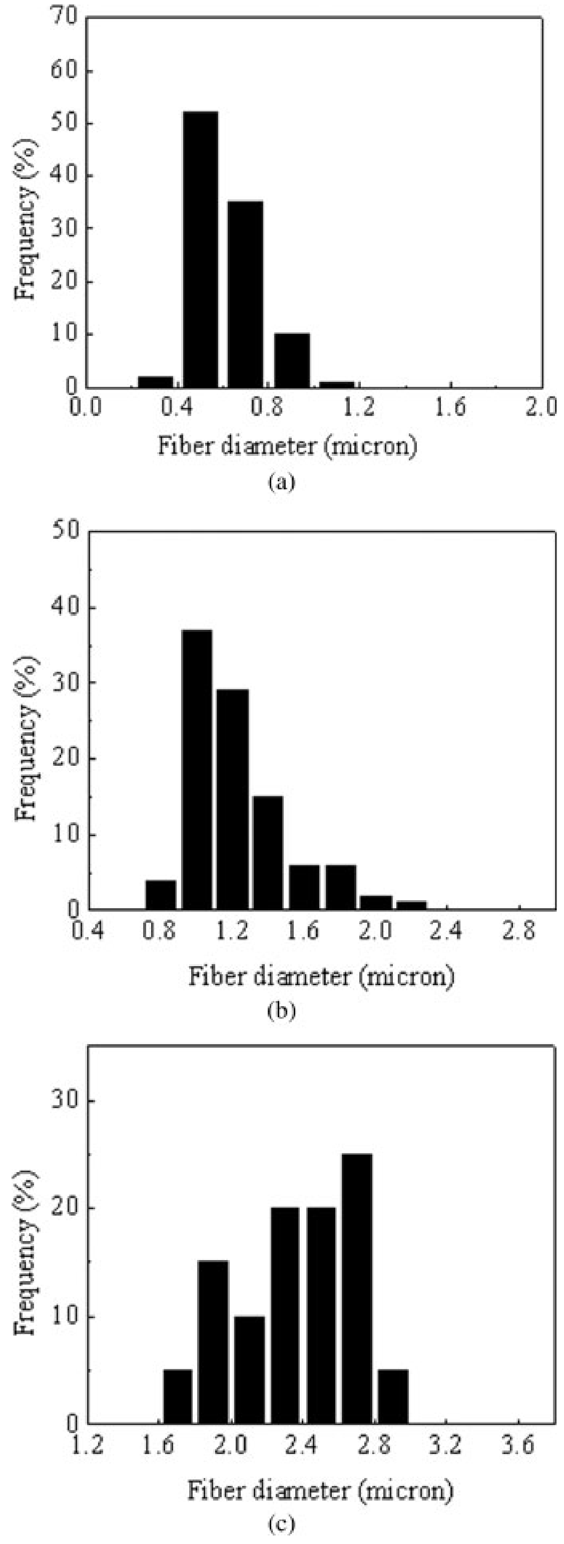

Figure 4 The fiber diameter distribution of PLLA/n-TCP composite continuous nanofiber yarns electrospun from PLLA/n-TCP solution with PLLA concentration of (a) 2.5% (w/v), (b) 5%, and (c) 7.5%, with PLLA/n-TCP weight ratio of 10/1. Conjugate electrospinning condition: power supplies, ±10 kV; distance between two spinnerets, 30 cm.

close nanofiber yarns are formed with the diameter about 150–200 μm [Fig. 3(c–f)], and the diameter of nanofiber is about 1–3 μm.

To explore the dispersion of n-TCP in the fibers, TEM measurement is performed with only one nanofiber electrospun from PLLA/n-TCP composite solution with PLLA concentration of 2.5% (w/v) and PLLA/n-TCP weight ratio of 10/1. We can observe nanoparticles dispersed in the fiber and cluster on the surface as shown in Figure 5. So, the rough surface of the as-spun nanofibers can be attributed to n-TCP on the surface.

Distance between two spinnerets

SEM images of PLLA/n-TCP composite continuous nanofiber yarns are shown in Figures 6 and 3(c,d) while changing the distance between two spinnerets from 20 to 40 cm. When the distance is 20 cm, we found that nanofibers bond each other to form tightly close yarn with the diameter of about 20 μm. As the distance increased, loose nanofiber yarns were formed.

The effect of distance between two spinnerets can be understood by the following argument. Electric field plays an important role on the initialing of polymer solution jet and resulting fiber morphology during electrospinning. In conjugate electrospinning, electric field will be increased while decreasing the

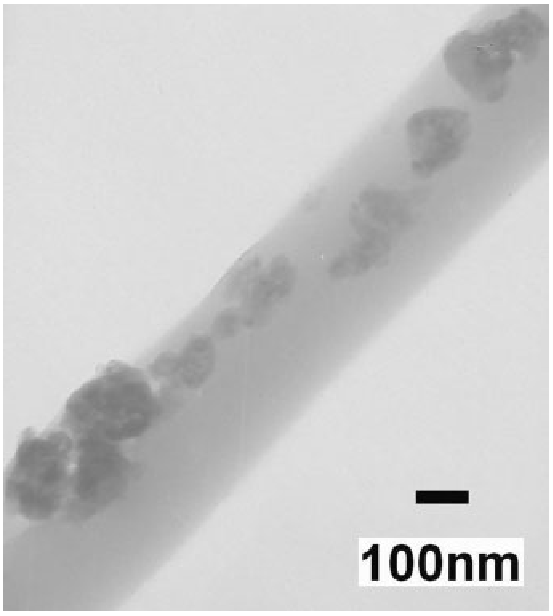

Figure 5 TEM image of nanofiber electrospun from PLLA/n-TCP solution with PLLA concentration of 2.5% (w/v) and PLLA/n-TCP weight ratio of 10/1. Conjugate electrospinning condition: power supplies, ±10 kV; distance between two spinnerets, 30 cm. Scale bar: 100 nm.

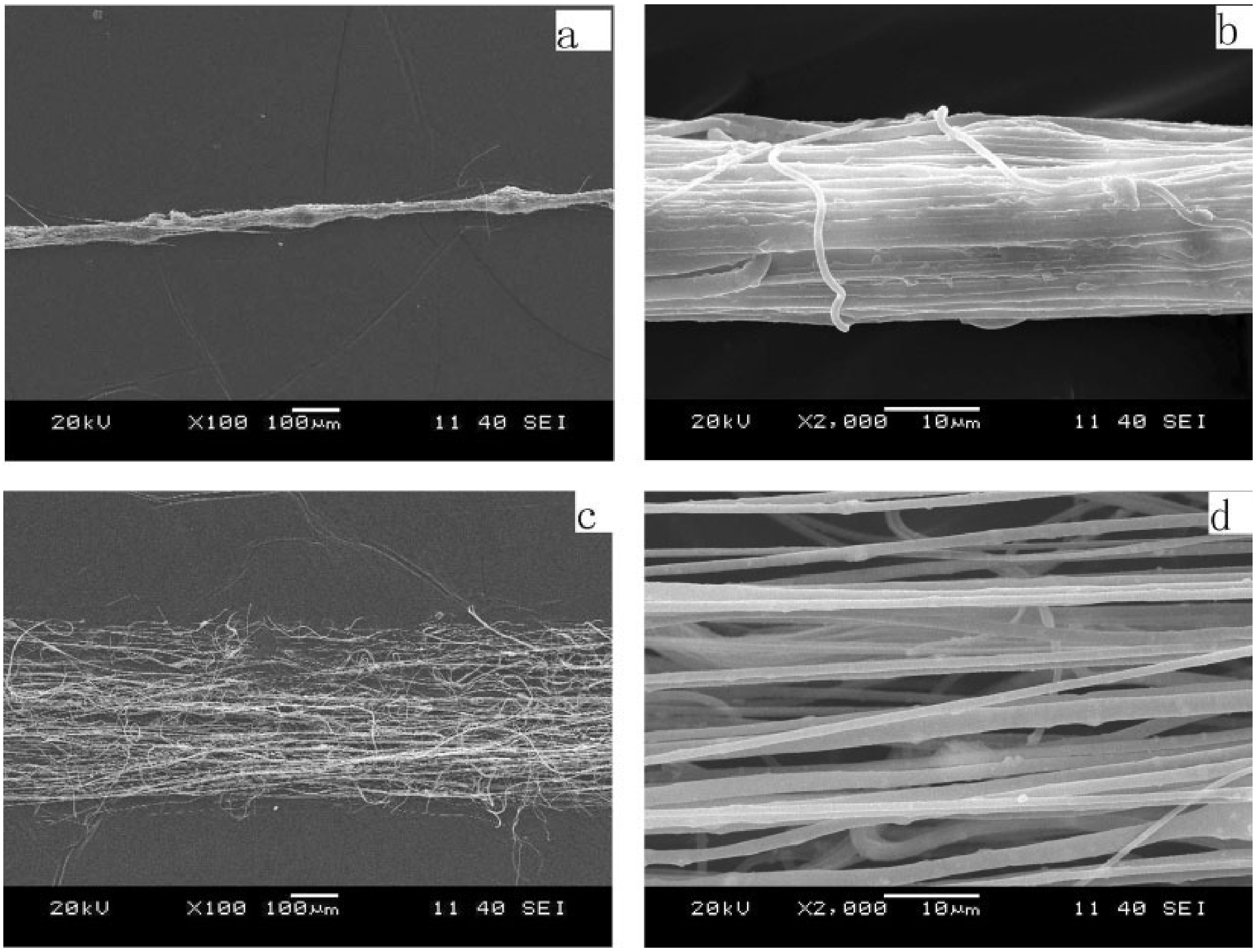

Figure 6 SEM images of PLLA/n-TCP composite continuous nanofiber yarns electrospun from PLLA/n-TCP solution with PLLA concentration of 5% (w/v) and PLLA/n-TCP weight ratio of 10/1. Conjugate electrospinning condition: power supplies, ±10 kV; distance between two spinnerets: (a, b) 20 cm, (c, d) 40 cm. Magnification: (a, c) 100; (b, d) 2000.

distance between two spinnerets. The decrease of the distance also decreases the journey of jet whipping before opposite charged fibers strike together, which will decreases the evaporation of solvent. So, the shorter distance will induce nanofibers bonding [Fig. 6(a,b)] and forming tightly close yarns with the smaller diameter. Longer distance increases the evaporation of solvent during conjugate electrospinning, and loose nanofiber yarns are formed as shown in Figure 6(c,d).

Content of n-TCP

While changing the content of TCP, nanofiber yarns can be obtained as shown in Figure 7. The results reveal that increasing the content of TCP does not induce obvious changes of morphologies of nanofibers, and the more the content of TCP, the more the roughness of surface of the nanofibers.

Multiple conjugate electrospinning of PLLA/n-TCP composite solution

To prepare PLLA/n-TCP composite nanofiber yarns with good mechanical properties, we extend conjugate electrospinning by using three pairs of spinnerets. The method is called multiple conjugate electrospinning, and the schematic experimental setup using three pairs of spinnerets is shown in Figure 2.

Two mixtures of PLLA/n-TCP composite are delivered to two columns of spinnerets at the flow rate of 15 mL/h for each side. Then two high electrical voltages of ±15 kV with opposite polarities are applied to two column spinnerets, respectively. We observed that mixture jets on the spinneret tips of two spinnerets are stretched out and do whipping movements under the action of the electrical field, resulting in the formation of charged nanofibers. The charged fibers attract each other to form nanofiber yarn. We notice that the nanofibers spun from the

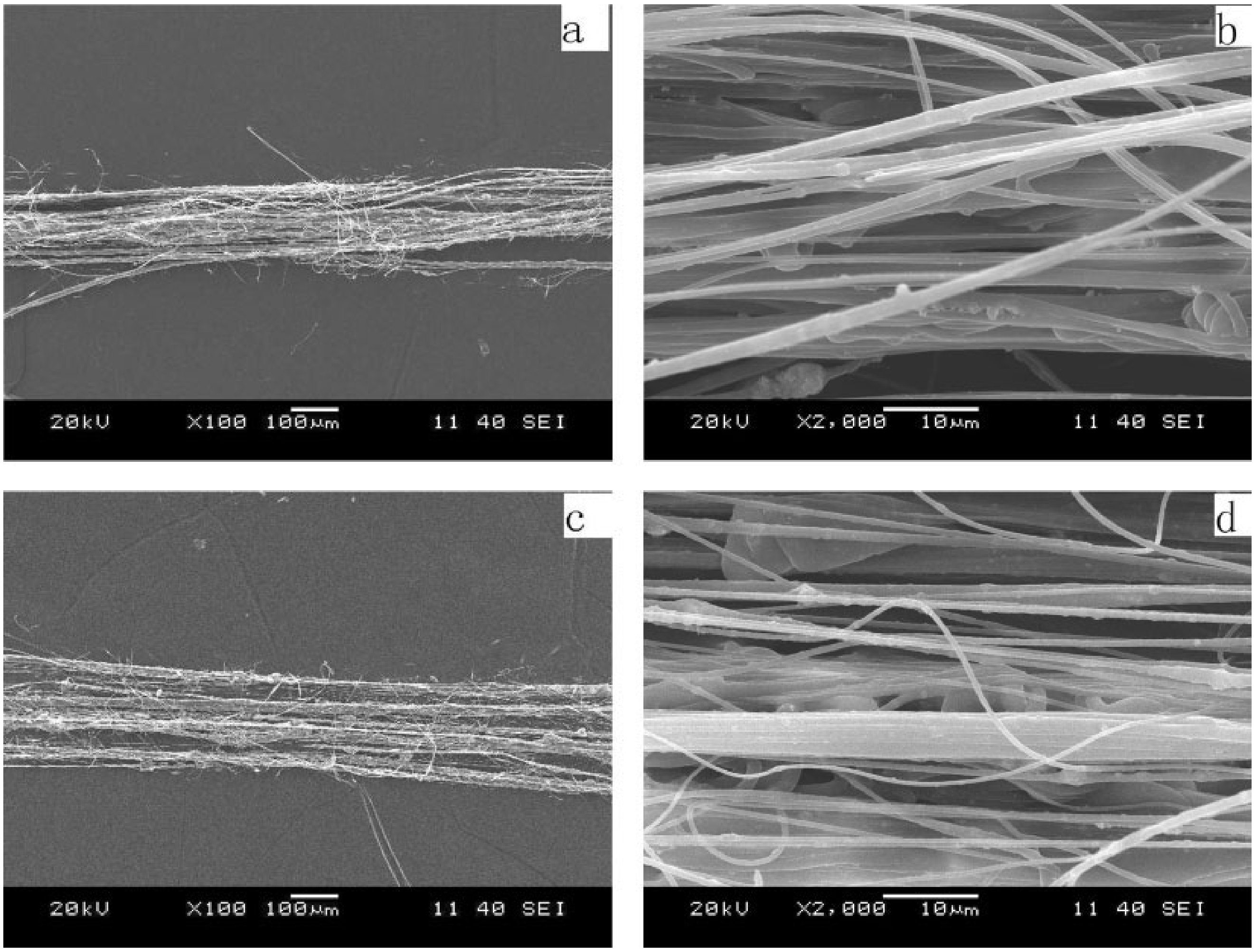

Figure 7 SEM images of PLLA/n-TCP composite continuous nanofiber yarns electrospun from PLLA/n-TCP solution with PLLA concentration of 5% (w/v) and PLLA/n-TCP weight ratio of (a, b) 20/1 and (c, d) 5/1. Conjugate electrospinning condition: power supplies, ±10 kV; distance between two spinnerets, 30 cm. Magnification: (a, c) 100; (b, d) 2000.

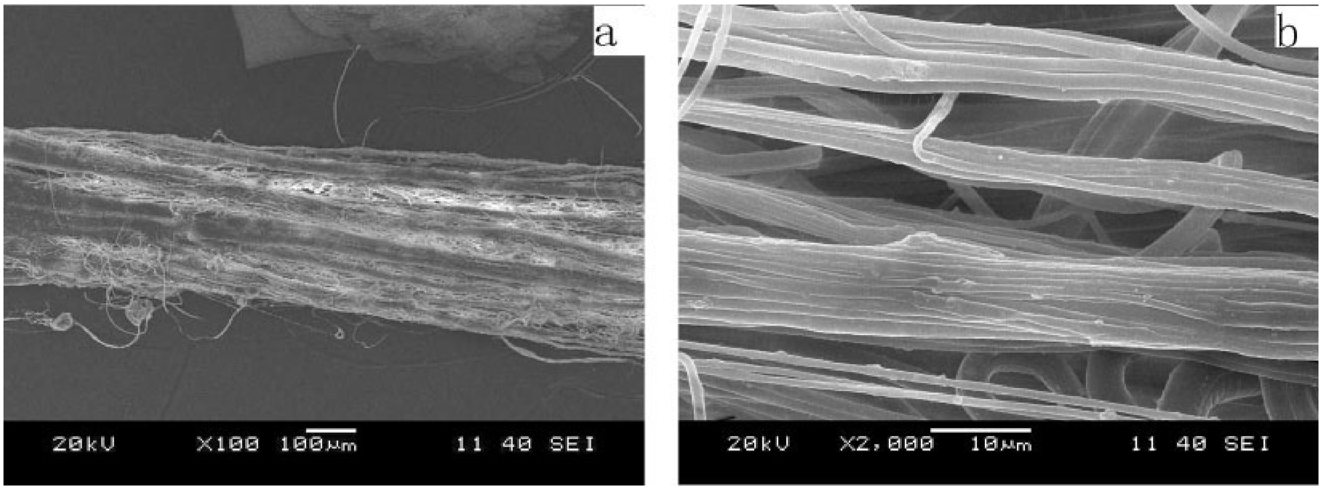

Figure 8 SEM images of PLLA/n-TCP composite continuous nanofiber yarns electrospun from PLLA/n-TCP solution with PLLA concentration of 5% (w/v) and PLLA/n-TCP weight ratio of 10/1. Multiple conjugate electrospinning condition: three pairs of spinnerets; power supplies, ±15 kV; distance between two spinnerets, 30 cm. Magnification: (a) 100; (b) 2000.

TABLE I
MTT Results of Electrospun PLLA/n-TCP Composite Nanofiber Yarns

Group	OD value*
Control	0.901 ± 0.038
A[a]	0.917 ± 0.030
B	0.924 ± 0.034
C	0.932 ± 0.065
D	0.927 ± 0.069
E	0.897 ± 0.046

* $P < 0.05$, $n = 5$.

[a] Nanofiber yarns electrospun from PLLA/n-TCP composite solution with PLLA concentration of (A) 2.5% (w/v), (C) 5%, and (E) 7.5%, with PLLA/n-TCP weight ratio of 10/1. Nanofiber yarns electrospun from PLLA/n-TCP composite solution with PLLA concentration of 5% (w/v), with PLLA/n-TCP weight ratio of (B) 20/1 and (D) 5/1. Conjugate electrospinning condition: power supplies, ±10 kV; distance between two spinnerets, 30 cm.

higher pair of spinnerets can attract nanofibers spun from the lower spinnerets. In other words, the nanofibers from the higher spinnerets can be regarded as the receiver of the fibers from the lower spinnerets. Therefore, thicker nanofiber yarn was obtained with some internanofiber bonding, as shown in Figure 8. The diameter of the yarn is up to 350 μm. The results indicate that nanofibers in the yarns align well, and each yarn also contains a large quantity of nanofibers with the diameter about 1 μm.

Mechanical properties of multiple yarns of PLLA/n-TCP composite nanofibers

The mechanical behavior of electrospun nanofiber yarns depends on the fiber structure. Especially, the geometrical arrangement of the fiber has an effect on the mechanical behavior. The mechanical properties of PLLA/n-TCP composite nanofiber yarns were measured by fiber tensile strength tester. We found that the nanofiber yarn of PLLA/n-TCP composite by conjugate electrospinning with one pair of spinnerets is too fragile to be handled, but single yarn of PLLA/n-TCP composite nanofibers electrospun from PLLA/n-TCP solution with PLLA concentration of 5% (w/v) and PLLA/n-TCP weight ratio of 10/1 by multiple conjugate electrospinning with three pairs of spinnerets has the tensile strength of 0.31 cN/dtex and elongation of 80.6%. The result reveals that PLLA/n-TCP composite nanofiber yarns may be knitted to prepare woven fabrics.

Cell compatibility of PLLA/n-TCP composite nanofiber yarns

The effect of PLLA/N-TCP composite nanofiber yarns on fibroblasts growth and proliferation determined by MTT assay is shown in Table I. The accuracy and reproducibility of the MTT assay have been evaluated, which requires only minimal processing and the results can be read on a standard multiwell scanning spectrophotometer. The OD values (optical density) of formazan converted from MTT is proportional to viability of cells and the ability of metabolically active cells. These data show that no measurable differences in OD values among any of the control case were detected. The results demonstrate that nanofiber yarns are not cytotoxic. The preliminary studies suggest that PLLA/n-TCP nanofiber yarns had good cell compatibility on the growth of rat fibroblasts, which gave us the promise of improving biocompatibility of nanofiber yarns a potential material for the fabrication of scaffolds after modification.

CONCLUSIONS

Nanofiber yarns of PLLA/n-TCP composite were prepared by conjugate electrospinning using a pair of two spinnerets arranged in opposite position, and applied with two high electrical voltages with opposite polarities. Majority of nanofibers in the yarns are oriented along longitudinal axis, which forms a unique aligned morphology. The concentration of PLLA had a significant impact on the morphology of nanofiber yarns. The nanofibers have rough surface, which is attributed to dispersion of TCP nanoparticles on the surface of the composite nanofiber. The distance between two opposite spinnerets has an effect on the evaporation of solvent during conjugate electrospinning, which may change internanofiber bonding.

By using three pairs of spinnerets, we developed multiple conjugate electrospinning. Thicker yarns can be obtained with nanofiber well aligned. The diameter of the yarn is up to 350 μm, and each yarn consists of a large quantity of nanofibers with the diameter about 1 μm. The yarns have tensile strength of 0.31 cN/dtex, which means the nanofiber yarns can be knitted to prepare woven fabric. Besides, the nanofiber yarn of PLLA/n-TCP composite exhibits good cell biocompatibility. The results suggest that nanofiber yarns may be woven to fabricate complex biodegradable scaffolds with high strength for tissue engineering.

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