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Short communication

Effects of solution properties and electric field on the electrospinning of hyaluronic acid

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ABSTRACT

In the present study, pure hyaluronic acid (HA) nanofibers were successfully fabricated at ambient temperature by electrospinning. HA powder was dissolved in a combination solution of deionized water, formic acid and DMF. The effect of the electrospinning solvent on electrospinnability was investigated in detail, the effect of the electric field between the needle capillary and a modified collector on the deposition of the charged fibers was discussed. The addition of formic acid greatly improved the electrospinnability of HA solution, pure HA nanofibers with a mean diameter below 100 nm were fabricated under the optimal condition.

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1. Introduction

Electrospinning has been generally accepted as a versatile and straightforward method for generating ultrafine fibers with diameters typically ranging from nano- to micrometers (Jalili, Morshed, & Ravandi, 2006; Li & Xia, 2004). Electrospun nanofibers have many amazing properties such as large surface area-to-volume ratio and high porosity with very small pore size, which have enabled nanofibers many applications that include filtration (Barhate & Ramakrishna, 2007), nanofiber reinforcement (Huang, Zhang, Ramakrishna, & Lim, 2004), tissue engineering (Ji, Ghost, Li, et al., 2006; Ji, Ghost, Shu, et al., 2006; Yoo, Lee, Yoon, & Park, 2005), wound healing (Rho et al., 2006) and release of drugs (Yang, Li, & Nie, 2007). During the process of electrospinning, the action of electrostatic force plays a vital role. When the electrostatic force at polymer droplet that suspended at the tip of the needle capillary overcomes the surface tension, a charged fluid jet is ejected towards the grounded collector. As the fluid jet travels through the air, the charged fluid jet undergoes a highly stretching and whipping process and the solvent evaporates quickly, as a result, continuous and ultrafine fibers deposited on the grounded collector that are typically randomly oriented in the form of nonwoven mats (Li, Wang, & Xia, 2003; Li, Wang, & Xia, 2004; Pan, Li, Hu, & Cui, 2006).

Hyaluronic acid (HA), a naturally occurring straight anionic polysaccharide, consists of repeating disaccharide units of (1-β-4)D-glucuronic acid and (1-β-3)*N*-acetyl-D-glucosamine (Fig. 1(a)), which widely exists in the extracellular matrix (ECM) of connective tissue (Meyer, 1947). Fabricating HA nano-scale fibers has attracted much attention for its remarkable applications in tissue engineering, wound healing and release of drugs due to its biodegradability, biocompatibility and wound healing ability (Ji, Ghost, Li, et al., 2006; Ji, Ghost, Shu, et al., 2006; Li, He, Han, et al., 2006; Li, He, Zheng, & Han, 2006; Xu et al., 2009). Heretofore, HA has been successfully electrospun by blending it with gelatin (Li, He, Han, et al., 2006; Li, He, Zheng, et al., 2006), PEO (Ji, Ghost, Li, et al., 2006; Ji, Ghost, Shu, et al., 2006), zein (Yao, Li, & Song, 2007), and collagen (Hsu, Huang, Liou, & Shen, 2010; Kim, Chung, & Park, 2008. Um, Fang, Hsiao, Okamoto, and Chu (2004) have reported that HA nanofibers were successfully prepared by electrospinning. In the process, a new air blowing-assisted setup is introduced to accelerate solvent evaporation rate and the air temperature is set at 57 °C. Li, He, Han, et al. (2006) and Li, He, Zheng, et al. (2006) have obtained HA nanofibers by electrospinning HA solution on aluminum plate that was immersed in coagulating bath of HA. The environmental temperature was controlled at 40 ± 3 °C, under which temperature the hydrogen bonds would be disrupted.

In this study, HA nanofibers have been fabricated by electrospinning at ambient temperature. A solvent was found to disrupt the hydrogen bonds in HA molecules to reduce the water retention ability of HA, a modified collector was introduced into the electrospinning process to assist the removal of the solvents. The influence

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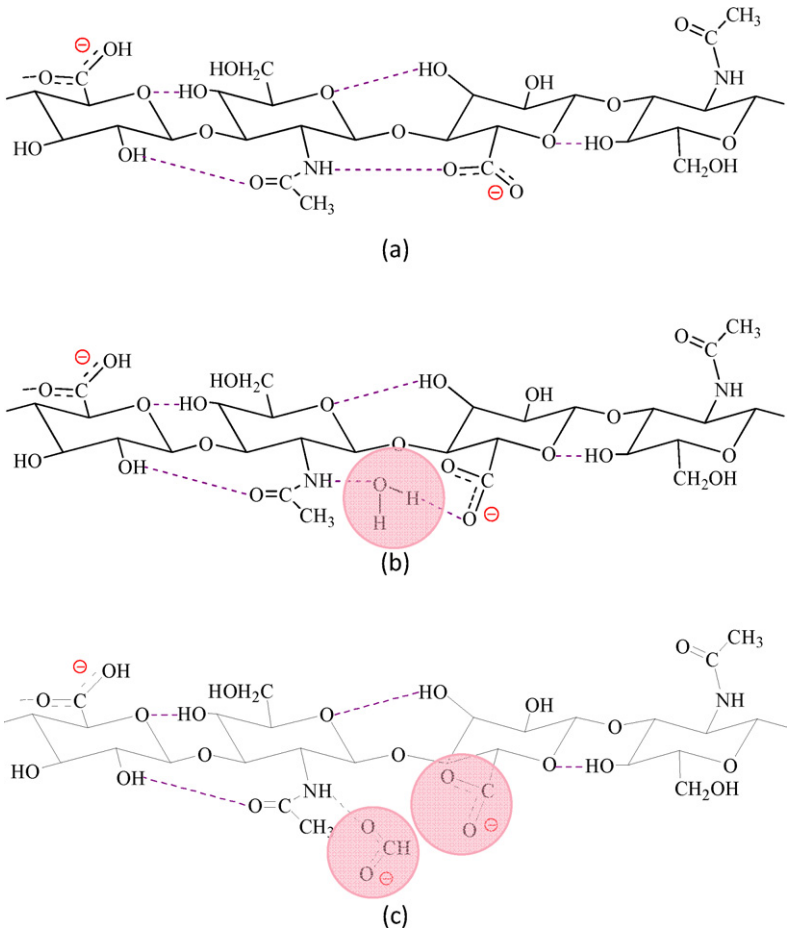

Fig. 1. (a) Chemical structure of HA. (b) Chemical structure of HA dissolved in DW. (c) Chemical structure of HA dissolved in DW/FA.

of solvents on HA solution properties and the electric field of the collector on the deposition of fibres were investigated.

2. Experimental

2.1. Materials

Hyaluronic acid used in this study was purchased from Dali Hyaluronic Acid Co., Ltd. of Liuzhou Chemical Group (Liuzhou, Guangxi, China) with the molecular weight of 1,000,000 g mol^{-1}. Formic acid (FA) and N,N-dimethylformamide (DMF) were supplied by Zhejiang Sunrise Chemicals Co., Ltd. (Zhejiang, China). All the materials were used without further purification.

2.2. Preparation of electrospinning solution

HA was added directly into a combination solution of deionized water, FA and DMF (25/50/25 by weight ratio) at ambient temperature with a weight concentration ranging from 0.8 wt% to 1.2 wt%, and then stirred sufficiently until HA dissolved completely. With the help of ultrasonication for 1 h, the air bubbles in HA solution were removed. A homogeneous and transparent HA solution was obtained.

2.3. Electrospinning

The electrospinning was done at ambient temperature (25 °C) and a relative humidity below 60%. The experimental apparatus of electrospinning is represented in Fig. 2. The solution for electrospinning was transferred into a 5 mL plastic syringe with a syringe pump (WSZ-50FZ, Zhejiang University Medical Instrument Co., Ltd.) at a constant rate of 0.3 mL h^{-1}. A modified aluminum foil-grounded collector (Fig. 2(a)) was employed to collect HA nanofibers. A high-voltage DC generator (BGG4-21, BMEI Co., Ltd.) was used to generate a constant positive high voltage (20 kV) between the tip of the needle capillary and collector (15 cm).

2.4. Characterization

The morphology of the electrospun HA fibrous membranes was characterized by field emission scanning electron microscopy (FE-SEM, S-4700, Hitachi) using an accelerating voltage of 20.0 kV. Each sample was sputtercoated with gold for analysis.

The conductivity of the HA solution was obtained through the conductivity meter (DDS-307, Rex Shanghai).

The surface tension of the HA solution was measured by a surface tension meter (DCAT21, EASTERN-DATAPHY).

The rheological property of HA solution was analyzed on a AR Rheometer (TA Instruments, USA) at 25 °C. Frequency sweeps were carried out for angular frequencies $\omega = 0.1–100$ rad s^{-1} at a strain amplitude of 1%. Shear measurements were performed in a range of shear rates from 0.05 to 90 s^{-1}.

3. Results and discussion

3.1. Properties of HA solutions

Hyaluronic acid (HA) can be dissolved in water, but HA nanofibers could not be obtained from electrospinning aqueous solution. In this study, a combination solution of deionized water

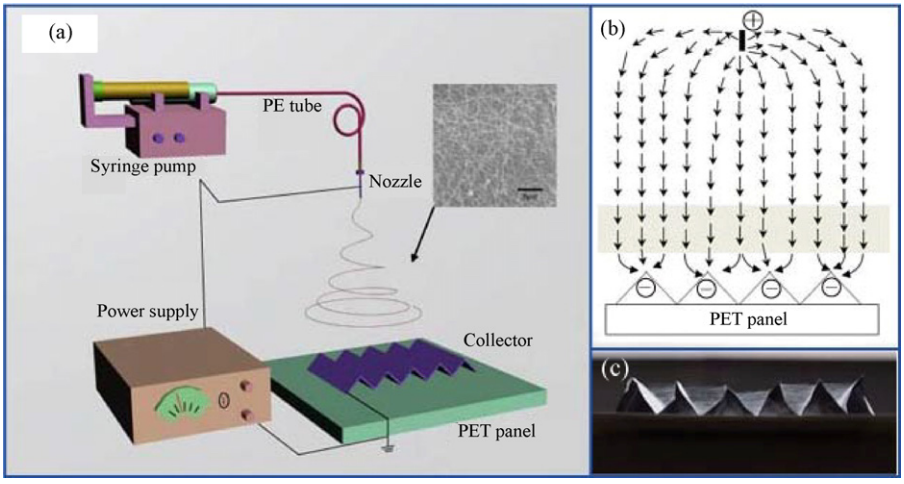

Fig. 2. (a) Schematic representation of the electrospinning setup that was used to fabric HA nanofibers. (b) Illustration of the electric field between needle and the modified collector. (c) Digital camera images of the arrangement of fibers deposited on the mountain-folded collector.

Table 1
Physical properties of HA solutions.

HA concentration (wt%)	WDW/WDMF/WFA	Conductivity (μS/cm)	Surface tension (mN/m)	Spinnability
1	100/0/0	1120	50.4	Negative
1	0/100/0	–[a]	–	–
1	0/0/100	–	–	–
1	50/50/0	3950	45.8	Negative
1	50/0/50	427	36.9	Negative
1	0/50/50	–	–	–
1	25/50/25	3910	40.3	Positive

[a] These results could not be obtained.

(DW), formic acid (FA) and DMF was used to dissolve HA powder. FA and DMF are used to improve the electrospinnability and the physical properties of HA solution.

Initially, HA powder was dissolved in the solvent of DW, FA, DMF, DW/FA, DW/DMF, FA/DMF and DW/FA/DMF, respectively. Electrospinning of these solutions was attempted at ambient temperature, a persistent and stable jet could be observed in the process of electrospinning only when DW/FA/DMF was used as the solvent. Following this discovery, we investigated the physical properties of the solutions with and without FA and DMF. Conductivity, surface tension and rheological measurement were carried out to understand the effect of FA and DMF on the properties of HA solution (Table 1).

As FA was introduced to the solution, the carboxylic acid group on FA would partially ionize, which results in the increase of ionic concentration and the improvement of solution conductivity (Table 1). The conductivity of the solution reflected the elongation level of the charged jet by electric force during the process of electrospinning. Therefore, the significant increase of conductivity may cause much higher elongation of the jet on an equal footing (Tan, Inai, Kotaki, & Ramakrishna, 2005). The viscosity of HA solution increased to a certain extent and the shear thinning behavior became more evident (Fig. 3) as FA was added. These effects could be explained from the fact that the inter- and intramolecularly hydrogen bonds among amide groups, carboxyl groups and hydroxyl groups (Fig. 1(b)) (Scott, Cummings, & Brass, 1991), which are the major contributing of the alpha helix structure of HA molecule, are disrupted to a certain extent by reconstructing hydrogen bonds with FA molecules (Fig. 1(c)). Therefore, the chain conformation of HA might be changed from the rigid alpha helix structure to the coil conformation, thus, the flexibility and the entanglements of HA chains are possibly improved. In addition, the surface tension measurement indicated that DMF could decrease the surface tension and conductivity significantly. With the reducing surface tension of solution, the formation of fluid jet would be observed at a relative low surface charge density.

3.2. Electrospinning of HA nanofibers

Among the solvents discussed above, the effect of HA concentration on the electrospinning process was investigated with various concentrations of 0.6 wt%, 1 wt%, and 1.2 wt% at a fixed volume ratio of DW/FA/DMF (25/50/25 by weight ratio). The ejected streams of

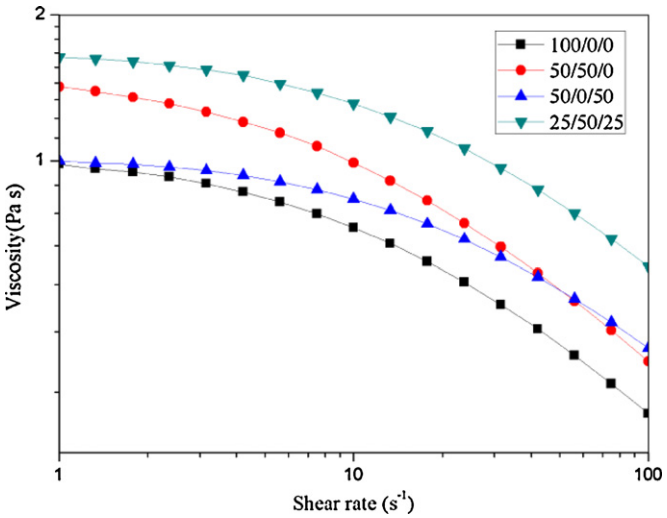

Fig. 3. Viscosity of 1 wt% HA solutions with different solvents, 100/0/0, 50/50/0, 50/0/50 and 25/50/25 represent the weight ratio of DW/FA/DMF.

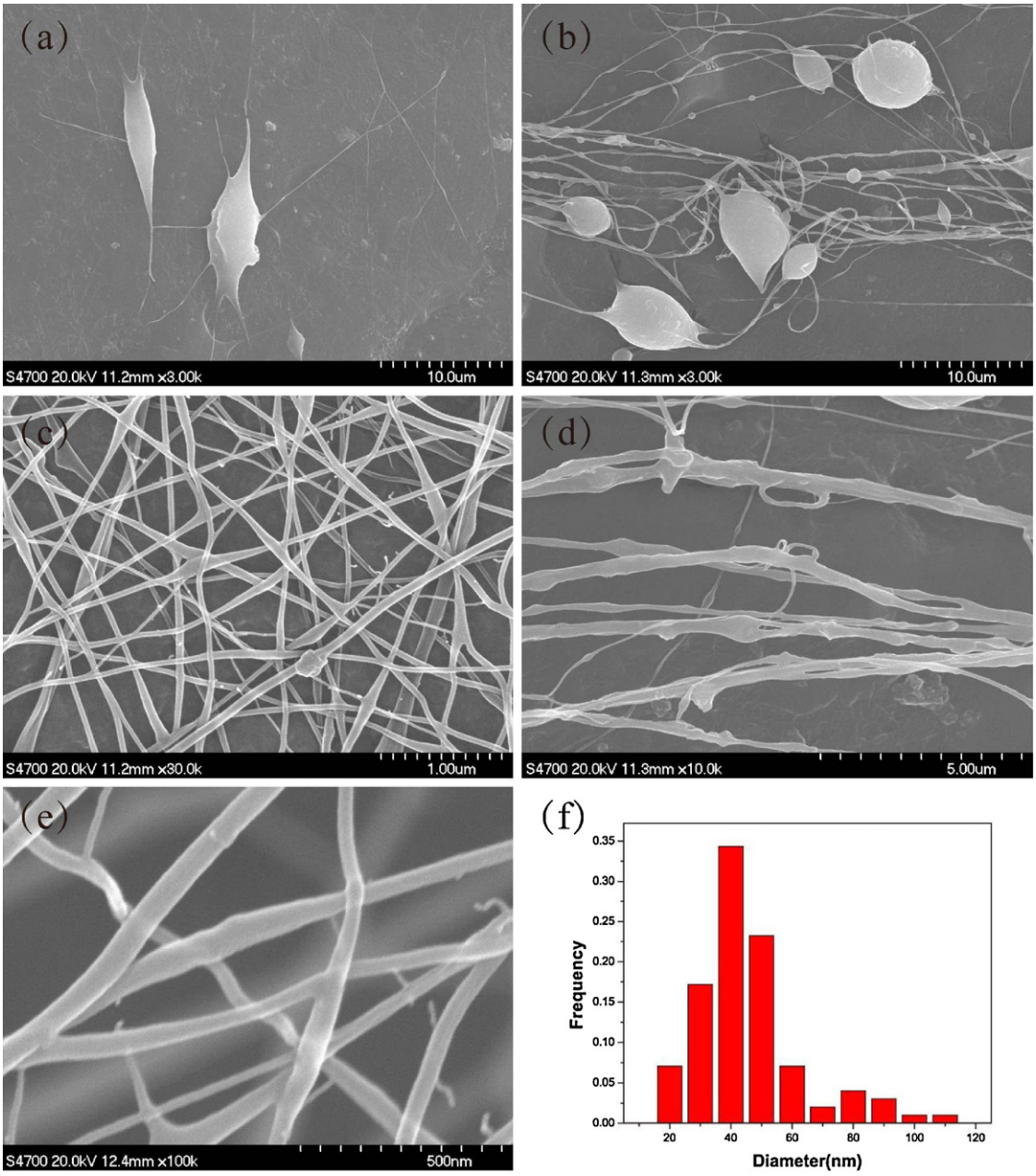

Fig. 4. SEM images of HA fibers electrospun from HA solution with different concentrations at room temperature. The voltage was fixed at 20 kV and the distance of tip to collector was fixed 15 cm. (a) 1 wt%, electrospun on the aluminum plate. (b) 0.6 wt%, electrospun on mountain-folded aluminum. (c) 1 wt%, electrospun on mountain-folded aluminum. (d) 1.2 wt%, electrospun on mountain-folded aluminum. (e) 1 wt%, electrospun on mountain-folded aluminum (100,000×). (f) The histogram of the fiber diameter distribution (1 wt%).

the solutions were continuous at all concentrations, but only the 1 wt% concentration solution could sustain a continuous jet for a comparatively long time. At 0.6 wt% concentration, a droplet was formed to be sprayed out every 2 min. When the HA concentration was increased to 1.2 wt%, clogging at the tip of the needle capillary was observed and interrupted the fiber formation in 1 min. Therefore, the electrospinning of HA solution was attempted at a specific concentration of 1 wt%. When HA was electrospun on an aluminum plate at room temperature, no fibers were observed on the aluminum plate, but the SEM image (Fig. 4(a)) showed that the ejected streams did form into fibers, which were fused to adjacent fibers and formed a film with few fibers and beads on the collector. The most likely explanation might be a result the solvents in the charged jet could not evaporate sufficiently before dried fibers could deposit on the aluminum plate.

Thus, in order to obtain dry fibers, a modified collector, mountain-folded aluminum foil (Fig. 2(c)), was incorporated into the electrospinning process, which was a simple and effective method to resolve the aforementioned problem. As shown in Fig. 4(c), pure HA nanofibers were obtained with diameters of tens of nanometres. It can be concluded that the electric field is distorted compared with the aluminum plate when the voltage is applied to the modified collector. For the charge density accumulation is related to the shape of the conductor surface, the charge density accumulated on the crests of the collector achieves the highest, but the charge density on the concaves close to zero. When the electric field lines approached to the crests of the collector, they were separated into several fractions pointing towards the crests of the collector Fig. 2(b). As the electrospun fibers access to the collector, the charged fibers will be induced towards the opposite charges

with higher charge density suspending across the concaves. Therefore, the modified collector enabled the fibers suspended across the concaves more surface area to expose to air, which speeded the evaporation rate of the solvents retained in the fibers. Li and Hsieh (2005) have revealed that the fibers deposited on the collector will not discharge immediately, which will cause Coulomb repulsion for the upcoming fibers. The Coulomb repulsion between the deposited HA fibers and the upcoming fibers avoided the fusion of the adjacent fibers, that is, with the aid of the Coulomb repulsion, more time was spared to assist the removal of the solvents from the electrospun fibers after they were deposited on the collector (Fig. 2(c)).

Distribution of fiber diameters calculated from SEM images using image analysis software indicated that the fiber diameter was comparatively small, over 70% of the fibers diameters distributed over a range from 30 nm to 50 nm (Fig. 4(f)).

4. Conclusions

Pure HA nanofibers could be prepared at room temperature by electrospinning HA solution. When HA powder was dissolved in the combination solution of DW, FA and DMF, the hydrogen bonds and the rigid alpha helix conformation of HA were partially disrupted. Rheological measurements indicated that FA could improve the HA chain entanglements by forming new hydrogen bonds with HA molecular after the hydrogen bonds and the rigid alpha helix conformation of HA were disrupted. As a modified collector was incorporated into the process, the electric field between the needle capillary and the collector was changed which facilitated the formation of pure HA nanofibers with a mean diameter below 100 nm.

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