

Environmentally benign synthesis of electrospun collagen-I in acetic acid solution

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Type-I collagen nanofibers were electrospun from aqueous collagen solution using acetic acid solution as a solvent, which is a replacement for 1,1,1,3,3,3 hexafluoro-2-propanol. A uniform nanofibrous mat of average fiber diameter of 120 nm was obtained from the following optimum condition: 14.3 mg/ml of collagen solution in aqueous 6% acetic acid solution with adding 10 wt% polyvinylpyrrolidone ethanol solution was successfully electrospun in the electric field of 17 kV. The aqueous acetic acid concentration higher than 2% was prerequisite for collagen nanofiber formation, because more concentrated acetic acid in water progressively decreased surface tension of the collagen solution and concomitantly increased charge density of jet without significant effect on solution viscosity. However, acetic acid solution more than 6% did not dissolve enough collagen to make spinnable viscous concentration. Only a mass ratio 1/5 of polyvinylpyrrolidone and collagen in the electrospinning solution produced bead-free collagen nanofibers, while low- or high-mass ratio of polyvinylpyrrolidone did not.

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1. Introduction

Bone-mimetic electrospun scaffolds consisting of collagen-I and nanoparticulate hydroxyapatite that mimic the nanoscale features of the extra cellular matrix have previously been promising for application as scaffolds for hard tissue regeneration. To date, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) appears to be the only solvent that can be used to electrospin collagen without the aid of carrier polymers [1-2]. Collagen type I, II and III can be solubilized in HFIP and electrospun from solutions at 0.05g/mL concentration and higher. Apparently, HFIP plays a dual role in collagen solubilization, with the two trifluoro-methyl groups serving to break hydrophobic interactions and the mildly acidic secondary alcohol hydroxyl assisting in breaking hydrogen bonding. However, Dimitrios I. Zeugolis et al show for the first time that the very properties that have established collagen as the leading natural biomaterial are lost when it is electrospun into nanofibers out of fluoroalcohols such as 1,1,1,3,3,3-hexafluoro-2-propanol. They further identify the use of fluoroalcohols as the major culprit in the process [3]. In addition, J. Lannutti have also indicated that type I collagen can be dissolved in hexafluoropropanol (HFP) and electrospun, and determined that the resulting nanofiber can contain, immediately after spinning, as much as 1600 ppm of residual HFP. Being the fluorinated analog of isopropanol, a common sterilizing agent, HFP certainly has the potential to negatively influence cell culture. Subsequent

treatment with vacuum, reduced HFP levels to well below 100 ppm; whether these levels are low enough to completely eliminate downstream biological effects is query [4]. Given the corrosive nature and toxicity of HFIP, it is desirable to find a more environmentally benign solvent or solvent system. Moreover, recent research has suggested that collagen fibers electrospun from HFIP lack native ultrastructure [5-6] in contrast to an earlier report, further encouraging the exploration of new solvents. We report here an acetic acid/ethanol system in which collagen is apparently soluble and from which collagen nanofibers can be electrospun by adding a certain amount of polyvinylpyrrolidone (PVP), which have not been reported yet.

2. Experimental

2.1. Materials

The collagen type I from calf skin (MW 300,000), PVP (MW 1,300,000 and MW 400,000), acetic acid (HAc, 99.8% AR, Tianjin Chem. Co., China), Ethanol Absolute (AR, Tianjin Chem. Co., China).

2.2 Preparation of spinning solutions and nanofibers

To obtain electrospinnable solutions, type I collagen (14.3 mg/ml) was dissolved in 6% acetic acid by magnetic

stirring to obtain a viscous solution, a certain amount of PVP powder was dissolved in ethanol absolute and vigorously stirred at room temperature to ensure homogeneity. The schematic setup of the electrospinning process used in this study was similar to those depicted elsewhere [7–10]. In brief, the above composite solution was placed into a plastic syringe equipped with a 7-gauge blunt end needle. The positive electrode of a high voltage power supply (High Static Voltage) was connected to the stainless steel needle by copper wires. The solutions were electrospun at 15–30 kV voltage and 15 cm working distance (the distance between the capillary tip and the collector). The electrospinning was performed in a vertical spinning configuration at room temperature with relative humidity of about 20%. The electrospun fibers were collected either directly on the aluminum foil or on foil paper. The scheme of the strategy for fabrication of collagen nanofibers was shown in Fig. 1.

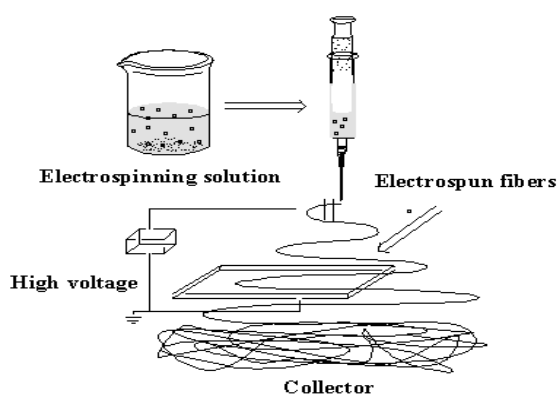


Fig. 1. Scheme of the strategy for fabrication of collagen nanofibers.

2.3 Characterization of electrospun fibers

The viscosity of precursor solutions was determined by using a digital viscometer at 25 °C (Brook-field Model DV III, USA). A static surface tension meter was used to measure the surface tension of electrospinning solution using Du Nouy ring method. The electrospun samples were dried under vacuum at 60 °C overnight to remove acetic acid and water possibly remaining after the electrospinning. Morphological characterization of the nanofibers was performed using a scanning electron microscope (SEM, JSM-5600LV, JEOL, Japan) with a beam voltage at 10kV. All samples were sputter-coated with gold before SEM observation. Statistical diameter distributions were obtained from at least 50 measurements based on the SEM images.

3. Results and discussions

3.1. Acetic acid concentration effect

Generally, surface tension determines the upper and lower boundaries of electrospinning window if all other

variables are held constant. The formation of droplets, bead and fibers can be driven by the surface tension of collagen solution and lower surface tension of the spinning solution helps electrospinning to occur at lower electrospinning voltage. Usually, collagen solution can be easily made by dilute aqueous acid solution such as 3% or higher and the collagen solution containing dilute acetic acid was not electrospinnable irrespective of collagen concentration. One of the most interesting results from our studies on the electrospun collagen/acetic acid system is that the acetic acid concentration in water strongly influenced surface tension of collagen solutions, which was remarkably important in collagen electrospinning. As acetic acid concentration increased from 1% to 8%, surface tension of 14.3 mg/ml collagen dissolved in different acetic acid concentrations with 10 wt% PVP (1,300,000 g/mol) decreased from 54.6 to 31.5 dyn/cm without significant viscosity change (Fig. 2).

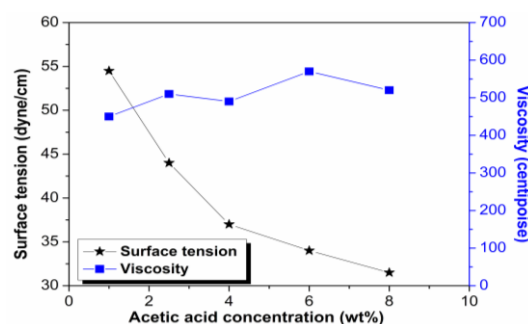


Fig. 2. Surface tension and viscosity of 14.3 mg/ml collagen dissolved in different acetic acid concentrations with 10 wt% PVP (1,300,000 g/mol).

Net charge density of the collagen solution also increased with increasing acetic acid concentration in water resulting in more charged ions available for charge repulsion. It has been known that salt addition in polymer solution increases charge density on the surface of ejected jet in electrospinning field and the higher charge density carried by the jet formed a smooth fiber more likely due to stronger whipping instability of the jet [11]. Among the acetic acid concentrations studied, 6% aqueous acetic acid was found to be most suitable for the formation of uniform nanofiber as shown in Fig. 3. At the acetic acid concentration of 3% or more, several thin nanofibers about 40 nm in average diameter were initially produced together with large beads, and at 6% acid concentration the fibers diameter increased to 100 nm without beads. However, neat acetic acid did not dissolve enough collagen to be electrospinnable. Therefore, the well known difficulty in the electrospinning of collagen nanofiber was solved by dissolving collagen in 6% acetic acid in water resulting in lowering surface tension and increased charge density of aqueous collagen/PVP solution. Therefore, the improved electrospinning behavior of the collagen solution can be attributed to finding of appropriate acetic acid concentration. Charge density of the jet at the same time

resulted in lower electric field required for jet formation.

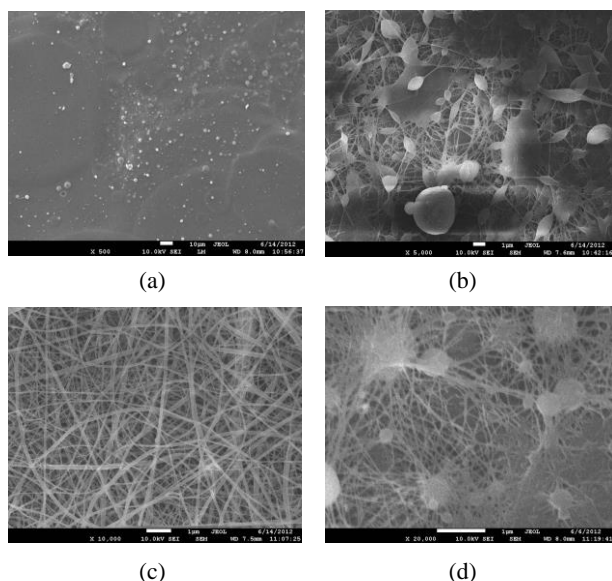


Fig. 3. SEM images of electrospun fibers at different acid concentration (wt%): (a) 1, (b) 3, (c) 6, (d) 8; 4 kV/cm, 10wt% PVP (1,300,000 g/mol). 17kv, 15cm.

3.2. Polyvinylpyrrolidone molecular weight effect

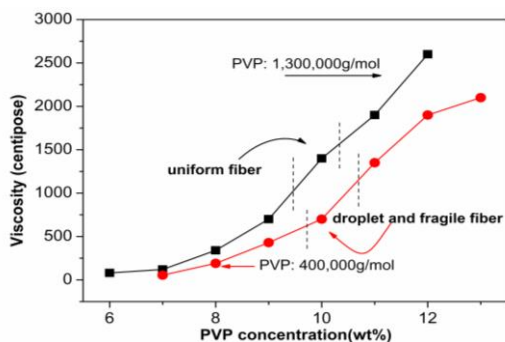


Fig. 4. Spinnability and relationship between concentration of PVP and viscosity of spinning solution. Best spinnable conditions were between the concentrations indicated by vertical lines.

In the electrospinning of certain molecular weight polymer, fibrous structure can be stabilized only above a critical concentration and below process limiting viscosity [12-13]. Molecular weight also has a significant effect on the viscosity, surface tension, conductivity and dielectric strength. It has been reported previously that too low molecular weight polymer tend to form beads rather than fibers and high molecular weight polymers give fibers with larger average diameter [14-15]. Two different molecular weights of PVP closely related to viscosity and spinnability as shown in Fig. 4.

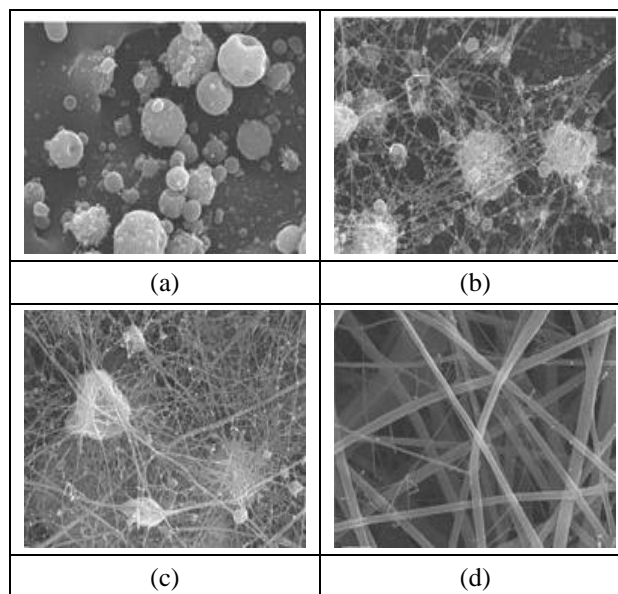


Fig. 5. SEM images of electrospun fiber at different molecular weights of PVP: (a) 400,000g/mol, 8%; (b) 400,000 g/mol, 10%; (c) 1,300,000 g/mol, 8%; (d) 1,300,000 g/mol, 10%; 6% acetic acid, 17 kV, and 15 cm.

Among two PVP with different molecular weights, only about 10% of 1,300,000 g/mol PVP (viscosity ranging from 1200 to 1500 cP) can produce a continuous and uniform fiber. The electrospun samples of the lower molecular weight 400,000 g/mol PVP solution (9.7–11%) usually contained large size beads and the fibers were fragile. Fig. 5(a, b, c, d) shows the fiber morphology of two kinds of PVP solution with similar viscosity at the most suitable spinning condition studied. With 400,000 g/mol PVP only fragile fibers with several droplets were obtained. Uniform nanofiber morphology was stabilized at molecular weight of 1,300,000 g/mol (average diameter of 100 nm). The large population of smaller fiber at the higher molecular weight PVP may be due to higher molecules of PVP having higher viscosity. Drastic morphological changes were observed when PVP concentration increased at the same molecular weight. With an increase in PVP concentration, morphology seemed to change gradually from a mixture of droplet and fiber, through uniform fiber to fibers with spindle-like beads. At 8 % concentration (Fig. 5(a)) chain entanglements may not be enough, resulting in spraying of large beads connected with very thin fibers. Fine fibers with some spindle-like beads were formed at 10% concentration (Fig. 5 (b)) in comparison with bead-free fibers at 10% sample (Fig. 5 (d)). Therefore 10% PVP concentration, at a molecular weight of 1,300,000 g/mol, was the most suitable for uniform and bead-free fiber spinning.

4. Conclusions

Several parameters including molecular weight of PVP, PVP concentration and acid concentration were investigated in the electrospinning of collagen solution dissolved in acetic acid solution. Among the parameters the acetic acid concentration was the most important parameter in the electrospinning, which decreased surface tension of the collagen solution and at the same time increased charge density of jet without significant effect on viscosity. The fiber morphology changed from beaded fiber to uniform fiber with increasing acetic acid concentration, implying significant influence of the concentrated acetic acid on reducing the applied field required for electrospinning. Only collagen dissolved in 6% aqueous acetic acid solution, with adding PVP solution of a molecular weight of 1,300,000 g/mol at 10% concentration, formed a bead-free and more uniform nanofibers.

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