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Preparation of gelatin/PVA nanofibers and their potential application in controlled release of drugs

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Abstract

Gelatin/PVA bicomponent nanofibers were prepared via electrospinning, and its control release of Raspberry ketone(RK) was investigated. Significant diameter increase, tensile strength and elongation at break improvement were observed as the ratio of PVA increased. The burst release of drug was observed in the first hour, and reached a plateau after two hours. The RK release rate could be tailored by the GEL/PVA ratio, the content of loaded RK, and the crosslinking time of glutaraldehyde vapor.

Keywords: Electrospinning; Gelatin; PVA; Raspberry ketone

1. Introduction

Within the last decade, the use of biodegradable polymers as effective carriers for drug delivery had found many applications in biomedical fields (Edwards et al., 1997; Jeong, Bae, Lee, & Kim, 1997; Kenawy et al., 2002; Moroni, Licht, Boer, Wijn, & Blitterswijk, 2006; Singh et al., 2001; Van et al., 2006). Compared to conventional dosage forms, polymeric drug delivery systems had many advantages, such as improved therapeutic effect, reduced toxicity, convenience, and so on (Chew, Wen, Yim, & Leong, 2005; Zeng et al., 2003; Zong et al., 2002).

Electrospinning is a process that an electric field is used to control the formation and deposition of polymers. When an electric field is applied between a needle capillary end and a collector, surface charge is induced on the polymer fluid deforming a spherical pendant droplet to a conical shape, so-called "Taylor cone". At a critical voltage, the electrostatic repulsion force of surface charges overcome surface tension and so the charged fluid jet is ejected from the tip of the Taylor cone. High surface charge densities enhance a whipping mode, where bending of the jet produces highly stretched polymeric fiber with simultaneous rapid evaporation of the solvent. The fibers are deposited on the collector in the form of non-woven membranes with individual fiber diameters that typically range from 100 nm to 1 μm. In the process of electrospinning, important parameters include electric voltage, spinning distance, inner diameter of the syringe needle, concentration of dope solution, viscosity, and conductivity (Doshi & Reneker, 1995; Reneker & Chun, 1996).

The fibers produced via electrospinning formed a porous network which had very large surface area-to-volume ratio and high porosity with very small pore size. Therefore, it could be very promising materials for many biomedical applications such as drug delivery, wound dressing, artificial organ and medical prostheses (Huang, Nagapudi, Apkarian, & Chaikof, 2001; Jin, Chen, Karageorgiou, Altman, & Kaplan, 2004; Li, Laurencin, Caterson, Tuan, & Ko, 2002; Matthews, Wnek, Simpson, & Bowlin, 2002; Min et al., 2004; Stitzel, Pawlowski, Wnek, Simpson, & Bowlin, 2001; Yoshimoto, Shin, Terai, & Vacanti, 2003).

Gelatin (GEL) is a natural biodegradable polymer derived from collagens and thus has almost the same

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composition and properties as those of collagens. Owning to its great biocompatibility and biodegradability, GEL was widely used as drug carriers nowadays (Dong, Wang, & Du, 2006; Kuijpers et al., 2000; Mohamad & Dashevsky, 2006; Van Den Bulcke et al., 2000). Huang et al. (Huang, Zhang, Ramakrishna, & Lim, 2004; Li et al., 2006; Zhang, Ouyang, Lim, Ramakrishna, & Huang, 2005) investigated the effect of mass concentration of dope solutions on the mechanical properties of GEL electrospun nanofiber nonwoven web. However, the study on electrospinning GEL nanofiber carriers for drug delivery was very limited.

Raspberry ketone (4-(4-hydroxyphenyl) butan-2-one; RK) is a major aromatic compound of red raspberry (*Rubus idaeus*). Raspberry contains an abundance of sugars, vitamins, minerals, and polyphenols. Recently, raspberry ketone was widely used as a fragrance in cosmetics and as a flavoring agent in foodstuffs, and the latest research approved that it could prevent and improve obesity and fatty liver through altering the lipid metabolism. More over, it had the properties of whitening and anti-inflammation to skin (Chie et al., 2005; Matsuji, Masashi, & Yoshio, 1988; Meij, Paulus, & Jong, 1994; Shida et al., 1988).

In this work, electrostatic spinning was employed to the preparation of RK-loaded nanofiber for potential use in drug administration. The mechanical properties and drug release properties were investigated as well.

2. Materials and methods

2.1. Materials

GEL of type A from porcine skin in powder form was purchased from Beijing Chemical Agent Co., its isoelectric point is 7–9 according to GB6783-94 (Beijing, China). PVA (degree of polymerization = 3500; 88% hydrolyzed) was obtained from Kuraray Co. (Osaka, Japan). RK with 99.9% purity was purchased from Takasago International Corporation (Tokyo, Japan). All other agents were analytically pure. The polymer and the solvent were used without further purification.

2.2. Electospinning

2.2.1. Electospinning of Gel/PVA dope solution

Gelatin was dissolved in formic acid (88 wt%) with concentration of 17 wt%, and 10 wt% PVA aqueous solution was then added into the gelatin solutions with Gel/PVA ratio of 10/0, 7/3, 5/5, 3/7, 1/9, and 0/10, respectively. The mixtures were stirred for 1 h at room temperature. The solutions were transferred to a 5 mL plastic syringe fitted with a needle (diameter = 0.47 mm) and set up in the electrospinning apparatus (BMEI Co., Beijing, China). A piece of aluminum foil, which was used as a collector and grounded, was located 10 cm apart from the capillary tip. Electric potential was controlled at 30 kV and the electrospinning was performed at room temperature.

2.2.2. Preparation of RK-loaded Gel/PVA composite membranes

RK was added into the previous Gel/PVA solution (Gel/PVA 7/3, 9/1) with 2 and 5 wt%, respectively. After electrospinning, the membranes with RK were crosslinked in glutaraldehyde vapor (samples were put on the tops of 50 wt% glutaraldehyde aqueous solution) for predetermined time at 25 °C(except mentioned specially, the crosslinking time was 2 h).

2.3. Characterization

2.3.1. Measurement of viscosity and conductivity

The viscosity and conductivity of the Gel/PVA blend solutions were measured by a rotational viscometer (NDJ-79, Shanghai Jichang Geology Instrument Co., Shanghai, China) and electric conductivity meter (DDB-6200, Shanghai Rex Xinjing Instrument Co., Shanghai, China), respectively.

2.3.2. Scanning electron microscopy

Morphologies of the electrospun nanofiber were observed by use of a scanning electron microscopy (SEM) (Hitachi S-450, Tamura, Japan) with an accelerating voltage of 20 kV and the obtained images were analyzed by Image Tool software, and totals of 50 counts were used to calculate the average diameter of nanofibers. Before SEM observation, all of the samples were sputter-coated with gold.

2.3.3. Analysis of mechanical behavior

Mechanical properties of electrospun fibrous membranes were determined with a uniaxial testing machine (INSTRON1185, Instron Co., Cambridge, USA) with the use of a 10-N load cell under a cross-head speed of 10 mm/min at 23 °C. All samples were cut into dog-bone shape with straight flange dimensions of 20×5 mm by a standard metal mould, and then the aluminum foil was carefully peeled off. The thicknesses of samples were measured with a digital micrometer having a precision of 1 μm. At least three samples were tested for each type of electrospun fibrous membranes (Zhang, Ouyang, Lim, Ramakrishna, & Huang, 2005). The machine-recorded data were used to process the tensile stress–strain curves of the specimens. The tensile strains were obtained by dividing the crosshead displacements with the original gauge length (20 mm).

2.4. In vitro release

0.2 g of the nanofibers GEL/PVA/RK membranes were first punched into 80 mL of sodium acetate and potassium dihydrogen phosphate buffer solution, the RK release studies were carried out at 37 °C and 100 rotation/min (rpm) in a thermostatical shaking incubator (HZ-9610K, Taicang instrument Co., Taicang, China). Samples of 5 mL were taken from the buffer solution after 10, 20, 30, 40, 50, 60, 80, 100, 120, 180, 240 and 300 min. After sampling, 5 mL

fresh buffer solution was added for continuing incubation. The amount of RK present in the release buffer was determined by a UV–vis spectrophotometer U-3010 (Hitachi, Tamura, Japan) at the wavelength of 275 nm. The results were presented in term of cumulative release as a function of release time:

$$\text{Cumulative amount of release } (\%) = \frac{M_t}{M_\infty} \times 100\%$$

where M_t was the amount of RK released at time t, the amount of RK added to electrospinning solution was regarded as M_∞ in this paper. Five samples were tested for each nanofibers membranes.

3. Results and discussion

3.1. Morphology of electrospun nanofibers

The morphological structures of electrospun Gel/PVA composite nanofibers are showed in Fig. 1. It was easy to find that the diameter of electrospun fiber increased as the ratio of PVA increase. Because increasing the ratio of PVA, the conductivity of the dope solution decreased (Table 1), the surface charge densities of the jet deceased, the electrostatic repulsion forces of the charged jet reduced. Thus the diameter of electrospun nanofibers tended to increase. On the other hand, as the ratio of PVA increase, the viscosity of dope solution increased (Table 1), which also inclined to obtain the fibers with large diameter. The properties of the solution, average diameter, and standard deviation of the nanofibers are shown in Table 1. The morphologies of GEL/PVA nanofibers with RK were also consistent with SEM observation before and after crosslinking (Fig. 1(h) and(i)). The membrane swelled obviously, and the boundaries of nanofibers were indistinct (Fig. 1(j)).

3.2. Mechanical properties

The tensile strength and elongation at break of the electrospun GEL/PVA nanofiber membranes with various ratios of PVA are showed in Table 2.

The results showed that the higher the ratio of PVA, the higher the tensile strength and the elongation at break for GEL/PVA nanofibers, because of the good deformability and flexibility of PVA. The miscibility leaded to difficult slippage of chains under loading because of more entanglements and strong physical interactions among the chains of mixed polymers, such as hydrogen bond. Thus, the addition of PVA was helpful to improve the mechanical properties of gelatin nanofiber membranes.

3.3. Drug release properties of RK loaded GEL/PVA nanofiber membranes

RK, GEL and PVA are water-soluble molecules, RK loaded GEL/PVA nanofibers could be easily dissolved in aqueous solutions, thus resulting in a large initial burst at

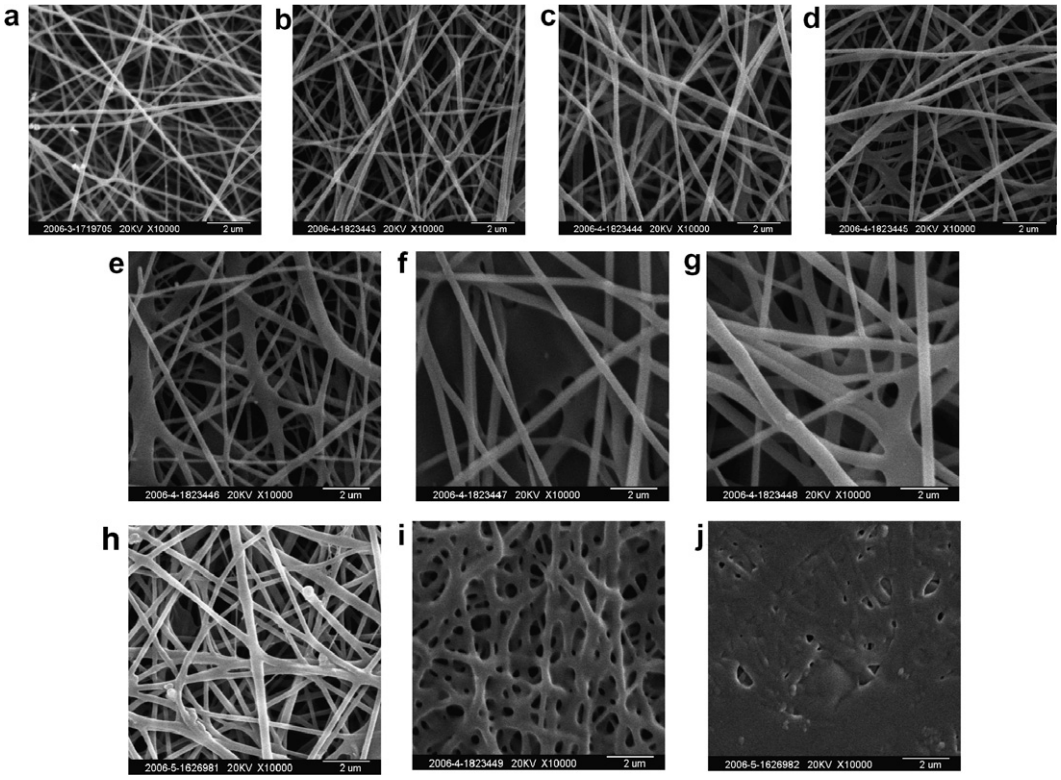

Fig. 1. SEM photographs of electrospun GEL/PVA nanofibers with various GEL/PVA ratios. (a) GEL:PVA = 10:0, (b) GEL:PVA = 9:1, (c) GEL:PVA = 7:3, (d) GEL:PVA = 5:5, (e) GEL:PVA = 3:7, (f) GEL:PVA = 1:9, (g) GEL:PVA = 0:10, (h) GEL:PVA = 7:3, RK = 5%, (i) membrane, (h) crosslinked by 50 wt% glutaraldehyde vapor for 5 h, (j) membrane, and (i) after drug release for 6h in buffer soltion.

Table 1
Properties of GEL/PVA solution and nanofibers

Ratio of PVA (%)	Conductivity (mS cm^{-1})	Viscosity (mPa s)	Average diameter (nm)	Standard deviation (nm)
0	5.50	400	133	28 [Fig. 1(a)]
10	5.44	750	177	38 [Fig. 1(b)]
30	5.22	830	212	36 [Fig. 1(c)]
50	5.03	1300	221	62 [Fig. 1(d)]
70	4.38	2100	273	92 [Fig. 1(e)]
90	2.93	2700	340	55 [Fig. 1(f)]
100	0.70	3000	447	129 [Fig. 1(g)]

Table 2
Properties of GEL/PVA electrospun fibrous membranes

Ratio of PVA (%)	Tensile strength (MPa)	Standard deviation (nm)	Elongation at break (%)
0	–[a]	–	–
10	2.4	0.1	4.0
30	4.7	0.3	5.0
50	6.3	0.1	20.0
70	8.6	0.4	25.0
100	11.6	0.6	60.0

[a] The mechanical property of the pure gelatin nanofiber membranes was very poor, and the samples were broken in the process of preparation and test.

short times. After two hours' of release, it reached a plateau. As swelling of the GEL/PVA nanofiber membranes in the buffer solutions, the inner drugs diffused to the buffer solutions through the carrier gradually and reached release equilibrium in the end. When the drug concentration was increased to 5%, the release percentage of RK was lower than the GEL/PVA scaffold with 2% of RK (Fig. 2).

3.3.1. Effect of pH value of the buffer solutions

There were both carboxyl and amino groups along the molecular chains of GEL, so GEL showed be sensitive to pH values of aqueous solution. Fig. 3 illustrates the release property of RK in different pH values of buffer solutions (pH 1, 4.8, and 7). In the buffer solution of pH 1, the RK release rate and final RK release percentage showed

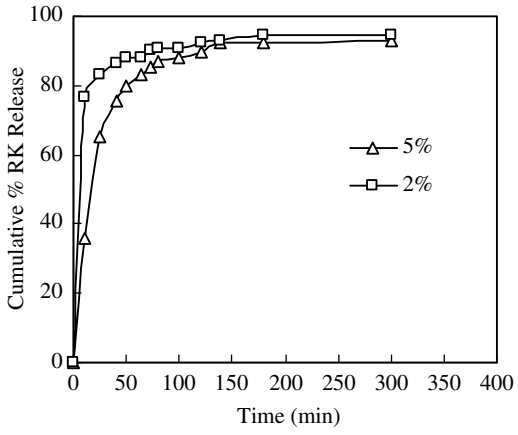

Fig. 2. The release percentage of RK in GEL/PVA (7.3) nanofiber membranes (pH 4.8, 37 °C).

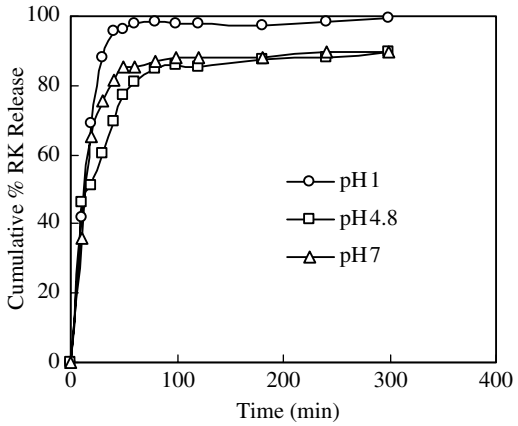

Fig. 3. Release percentage of RK (5%) from electrospun Gel/PVA (9:1) fibers with various pH values of buffer solutions (37 °C).

obviously faster and higher than that in the buffer solution of pH 4.8 and pH 7. The possible reason was when the pH value of the buffer solution was for less than the isoelectric point (PI) of GEL (PI 7–9), the amino groups were protonized to carry positive charges. Thus the whole polymer network carried positive charges which made molecular chains repulsed to each other. The network became looser and it was easy for the drug molecules to diffuse into the buffer solutions. The RK release properties of pH 4.8 (close to pH.value of human skin) and pH 7 (close to GEL isoelectric point) were similar because their swelling properties were very close.

3.3.2. Effect of crosslinking time

The poor water resistance and low mechanical strength of GEL nanofibers had limited its applications. However, its mechanical properties could be improved by the addition of PVA or chemical crosslinking through covalent bonds between the reactive side groups presents in gelatin and PVA. Glutaraldehyde has been used extensively because it has the advantage of being a fast-acting hardener for gelation (less than 1 min are reported for glutaraldehyde concentrations between 10 and 20 wt%), easily

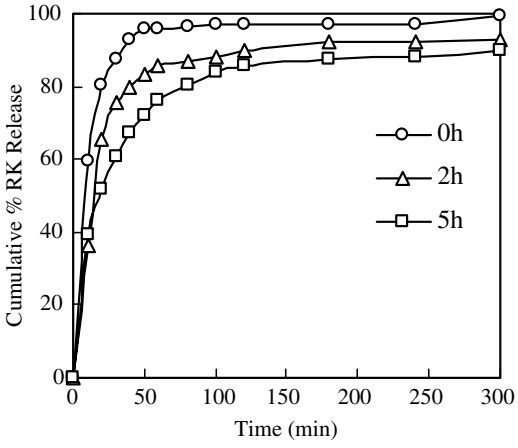

Fig. 4. Release percentage of RK (5%) from electrospun Gel/PVA (7:3) fibers with different crosslinking time (pH 4.8, 37 °C).

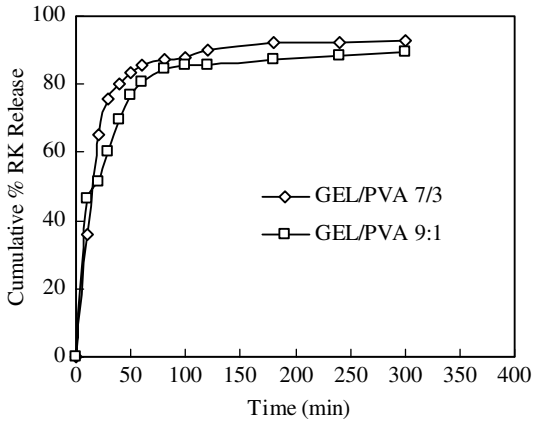

Fig. 5. Release percentage of RK (5%) from electrospun Gel/PVA fibers with different Gel/PVA mixture ratios (pH 4.8, 37 °C).

available and inexpensive (Chiellini, Cinelli, Fernandes, Kenawy, & Lazzeri, 2001).

Fig. 4 shows the RK release property of the membranes at different crosslinking time by glutaraldehyde. As the increase of crosslinking time, the reaction took place from the surface to the inside of the membranes, and the degree of crosslinking increased, the intermolecular forces enhanced, resulting in the slowing down of the swelling rate, and the speed of drug delivery.

3.3.3. Effect of GEL/PVA mixture ratios

Fig. 5 shows the results of RK release percentage of different ratios of GEL and PVA. The GEL/PVA = 7/3 nanofiber membranes had a slightly higher release rate than that of GEL/PVA = 9/1. Due to PVA solubility was better than GEL in the buffer solution, so the swelling degree of GEL/PVA(7/3) nanofiber membrane was slightly higher than that of GEL/PVA(9/1), and its release rate higher.

4. Conclusions

The RK loaded GEL/PVA electrospinning nanofibers should have potential application in controlled drug delivery based on the following observations: (1) The addition of PVA helped to enhance both the tensile strength and elongation at break of the membrane and (2) the RK release rate could be tailored by changing the content of RK in GEL/PVA matrix, the ratio of GEL and PVA, and the crosslinking time by glutaraldehyde vapor.

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