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MEDICAL POLYMERS

Preparation and Drug Release of PVA Composite Nanofibers Loaded Chitosan Microsphere[1]

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Abstract—In this paper, we reported the fabrication of poly(vinyl alcohol)-chitosan (PVA-CS) microspheres composite nanofibers by electrospinning technique. The chitosan microspheres were firstly prepared by electrospray with the solution of chitosan and combretastatin A4. The morphology and size distribution of chitosan microspheres were analyzed by scanning electron microscopy. The influencing factors including the concentrations of both PVA and CS microspheres were studied. The physical properties of the composite nanofibers were characterized by X-ray diffraction (XRD). The drug release rate, MTT toxicity test, and cell culture were also investigated in detail. Results indicate that the chitosan microsphere-loaded composite nanofibers can be prepared when the PVA concentration is 120 mg/mL. The continuity of the nanofibers was influenced by the concentration of CS microspheres. The characteristic peaks of CS or PVA were not observed in the diffractograms after the CS and PVA were processed using the high-voltage electrostatic technique. In addition, the drug release rate showed that nanofibers induce an obvious slow-release effect. Composite nanofibers were non-toxic to fibroblasts cells, and the fibroblasts cells could proliferate on the nanofiber mat.

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INTRODUCTION

The entrapment of drugs in polymer microspheres continues to be of interest in new drug formulation strategies. Compared with conventional drug formulations, microspheres with drug entrapped in a polymer matrix allow better control of delivery particularly for large molecules such as proteins and nucleic acids with low aqueous solubility [1]. In recent years, nanofibers have been used to achieve different controlled drug release profiles, such as immediate, pulsatile, delayed, sustained, and biphasic release. Among these profiles, sustained drug release is gaining considerable attention as a method of administering and maintaining desired drug concentrations in the blood within a desired duration of drug delivery [2].

There are several established technologies for producing microspheres and nanofibers. Some researches were focused on generate microspheres by cross-linking [3], precipitation [4], self-assembly [5, 6] and spray-drying [7] methods. Melt-spinning [8], dry-wet spinning [9] and sol-gel process [10] are always used to generate nanofibers. However, high-voltage electrostatic technology, an environmentally friendly technique, can be used to manufacture microspheres and nanofibers simultaneously. When the products were microspheres, the technique was known as "electrospray". Pérez-Masiá et al. prepared microspheres successfully with low-molecular-weight carbohydrate polymers by modulating the electrospraying conditions [11].On the other hand, when the products were nanofibers, the technique was known as electrospinning. For instance, hyaluronic acid (HA)-based nanofibers have been fabricated by electrospinning at ambient temperatures [12]. Electrospray and electrospinning have been recently suggested as a simple and straightforward method to generate submicron encapsulation structures. Furthermore, the use of water as solution does not generate toxicity problems [11]. In recent years, electrospray and electrospinning have attracted great attention due to their convenience, environmental friendliness, and safety. Wang et al. fabricated core-shell-structured nanofibers by electrospinning. The core-shell scaffold can be a good candidate for a biomaterial application in intravascular stents [13]. Among the variety of materials available to biomedical engineers, two of the most widely used are

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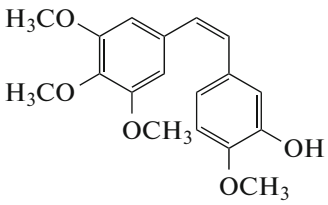
chitosan (CS) and poly(vinyl alcohol) (PVA). Chitosan is a natural biodegradable polymer produced from chitin deacetylation. In addition, its positive charge may overcome cellular barriers to enhance drug absorption [14]. PVA is a semi-crystalline and hydrophilic polymer, and it can be electrospun into nanofibers in aqueous solution [15]. Both compounds are widely used carrier materials in drug delivery due to their biocompatibility, bioadhesive properties, and degradability in the body. They have been studied extensively in recent years.

This work can be divided into two parts. In the first part, the chitosan drug carrying microspheres were produced by the electrospray technology. The drug is combretastatin A4 (CA4), which is a type of antiangiogenesis hydrophobic drug [16]. In the second part, chitosan drug carrying microspheres were added to PVA nanofibers by electrospinning technology. The surface morphology of the microspheres and nanofibers were firstly investigated. Then the internal structure and composition of the nanofibers transmission were analyzed by means of electron microscopy (TEM) and X-ray diffraction (XRD). Finally, the drug loading, the efficiency of drug loading and encapsulation and drug release rate of the nanofibers were researched in vitro.MTT toxicity test was employed to investigate the toxic, and cell culture was also studied in detail.

EXPERIMENTAL

Materials

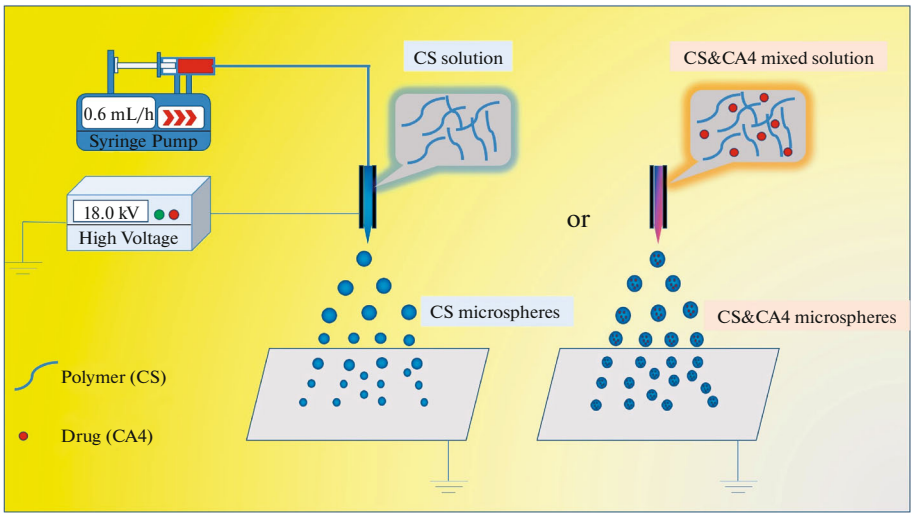
Chitosan (degree of deacetylation 0.8, $M_w = 1.2 \times 10^5$) and polyvinyl alcohol (PVA) were purchased from Fu Ruida pharmaceutical company, China. Combretastain A4 (CA4):



was purchased from Rui Shu biochemical company, China. Acetic acid (AcOH), anhydrous ethanol (EtOH), disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), potassium chloride (KCl), sodium dodecyl sulfate (SDS), liquid paraffin, petroleum ether, twain-80 and glutaraldehyde were purchased from Yong Hua fine chemicals company, China.

Solutions Preparation

The 50 mg/mL CS solution was prepared by dissolving CS in composite solvent: AcOH : distilled water : EtOH at volume ratio of 7 : 2 : 1. The chitosan solution could be electrosprayed to get the chitosan microsphere. CA4 was dissolved in EtOH at the concentration of 3 mg/mL. The CS and CA4 solutions were mixed at different weight ratio to obtain the homogeneous and transparent mixed solution, which prepared for the electrospray to get the chitosan microsphere loaded by CA4 according to the scheme:



PVA was added directly into deionized water at 90°C for stirring for 3h to obtain a weight concentration of 100, 120 and 150 mg/mL. Then chitosan microspheres were placed to PVA solution at the quality of PVA of 15, 30, and 45 wt % respectively, stirred sufficiently until a homogeneous suspension liquid was obtained. The grounded collector was a kind of composite solvent prepared by mixing of liquid paraf-

fin, petroleum ether and twain-80 at weight ratio 14 : 10 : 1. In addition, glutaraldehyde was added to the composite solvent in the dosage of 10 drops.

Electrospraying and Electrospinning

The prepared solutions were supplied into a 5mL syringe with a metal capillary needle (inner diameter = 0.5 mm). The solutions were injected from the syringe pump with feed rate of 0.2 mL/h in electrospraying and 0.5 mL/h in electrospinning respectively. The distance between the capillary assembly and substrate was approximate 15 cm and the applied voltage ranged between 19 and 20 kV. All experiments were conducted at room temperature.

Structure Characterizations

The surface morphology of the microspheres and nanofibers was characterized using a Hitachi S-4700 scanning electron microscopy (Hitachi Company, Japan). The internal structure of nanofibers was characterized by a Hitachi s-800 transmission electron microscopy (Hitachi Company, Japan). The ATR–FTIR spectra of samples were carried out on Nicolet 5700 FTIR spectrometer (Thermo Electron Corporation, Waltham, MA, USA). Spectra were collected at wavelengths in 400–4000 cm^{-1} by cumulating 64 scans at a resolution of 2 cm^{-1}. The wide-angle X-ray diffraction analyzer (Rigaku Co., Japan) was employed to obtain the XRD patterns by the reflection method with nickel-filtered CuK_α radiation ($\lambda = 0.154$ nm) at 40 kV and 100 mA between 5° and 40°.

Evaluation of Drug Entrapment in Nanofibers

CA4 loading content and entrapment efficiency in nanofibers were determined by disintegration of samples in 40 mL of solvent which mixed by AcOH, distilled water and EtOH at volume ratio of 7 : 2 : 1. Then 160 mL of PBS (pH = 6.8, added SDS with 0.05 mg/mL) was added to it and stirred for 2 h. 40 mg composite nanofibers membranes were immersed in 200 mL PBS solution. The amounts of CA4 were quantified by a UV–Vis spectrophotometer at a wavelength of 295 nm. All experiments were run in triplicate per sample. The CA4 loading content and entrapment efficiency were calculated using the following equations:

$$\text{CA4 content} = \frac{\text{amount of loaded CA4}}{\text{amount of added polymer and CA4}} \times 100\%$$

$$\text{Entrapment Efficiency} = \frac{\text{amount of loaded CA4}}{\text{amount of added CA4}} \times 100\%.$$

In vitro Drug Release Study

The in vitro drug release of CA4/PVA-CS composite nanofibers was studied by the dynamic dialysis method in PBS. The 40 mg samples were placed into Visking dialysis tubing (Millipore, molecular weight cutoff 8 kDa) and dialyzed against above phosphate buffer sat at 37°C in water bath shaker (Jin Tan science analysis instrument company, China) 100 rpm. At designated time intervals, the release media were collected and the fresh release media were added. The amounts of CA4 were determined by a UV–Vis spectrophotometer at a wavelength of 295 nm. All drug release experiments were run in triplicate per sample.

MTT Assay, Cell Apoptosis Analysis

MTT assay of nanofibrous mats were measured according to previous [17]. The amount of formazan was proportional to the quantity of viable cells. Briefly, fibroblasts cells (L929 cells) cultured with the pre-extracted medium of nanofibrous mats (1 × 1 cm) in the culture plat were washed gently with PBS to remove untransformed solutions and the residues of sample extractions. Then 25 μL MTT was added to each plate and the incubation temperature was 37°C for 4 h. Subsequently, DMSO (150 μL) was added to each plate to dissolve the MTT formazan purple crystals for 10 min. At last, the absorbency of the solution was measured at 490 nm by using an enzyme linked immunosorbent assay Reader (MODEL550, Bio-Rad, USA). The nanofibrous mats with cultured cells were fixed by 4% PFA in PBS for 10 minutes, permeabilized by 0.2% Triton X-100 in PBS for 10 minutes, stained with 200 ng/mL 4',6-diamidino-2-phenylindole (DAPI) for 5 minutes in the dark, and then washed with PBS for three times. The membranes were examined by a fluorescent microscope (Leica DM4000B).

RESULTS AND DISCUSSION

Morphology of Microspheres and Nanofibrous Mats

Although there are many parameters in electrospraying (e.g. flow rate, applied electric field, and the distance between needle and collector) that can be used to control the microsphere morphology and diameter, our previous study showed that 50 mg/mL CS with 15 wt % CA4 could produce excellent CS microspheres. As the scanning electron microscopy (SEM) image in Fig. 1 shows, the CS microspheres are uniform and smooth. After random selection and statistical analysis, the average size of CS microspheres was found to be only 0.31 μm. The electrospraying of all chitosan solutions was performed under the same processing conditions. The flow rate, applied voltage, and distance between needle and collector were set at 0.2 mL/h, 20 kV, and 15 cm, respectively.

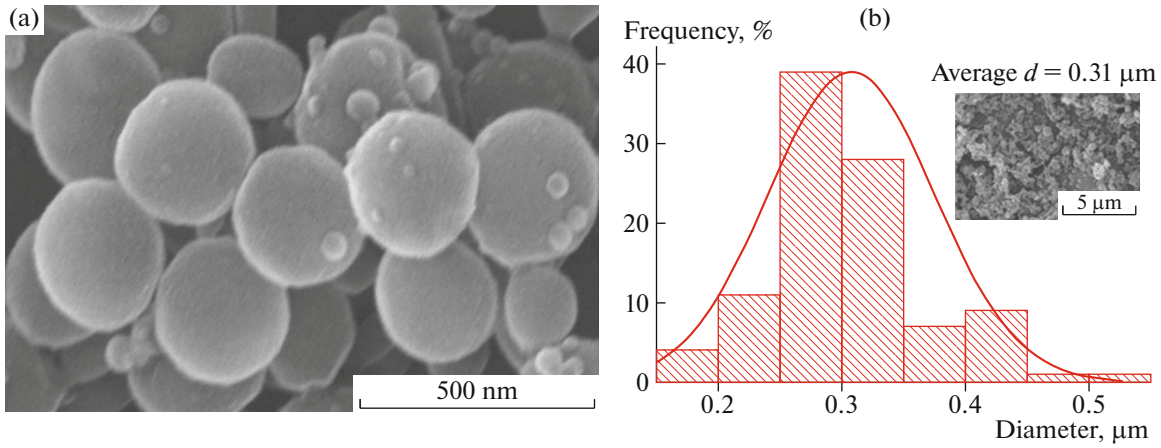


Fig. 1. (Color online) (a) SEM micrographs and (b) microsphere size distribution images of CS microspheres produced from 50 mg/mL CS-15 wt % CA4 solution. The solvent, flow rate, applied voltage and distance between needle and collector were set at AcOH/H_2O/EtOH (7/2/1), 0.2 mL/h, 20 kV and 15 cm, respectively. The grounded collector was liquid.

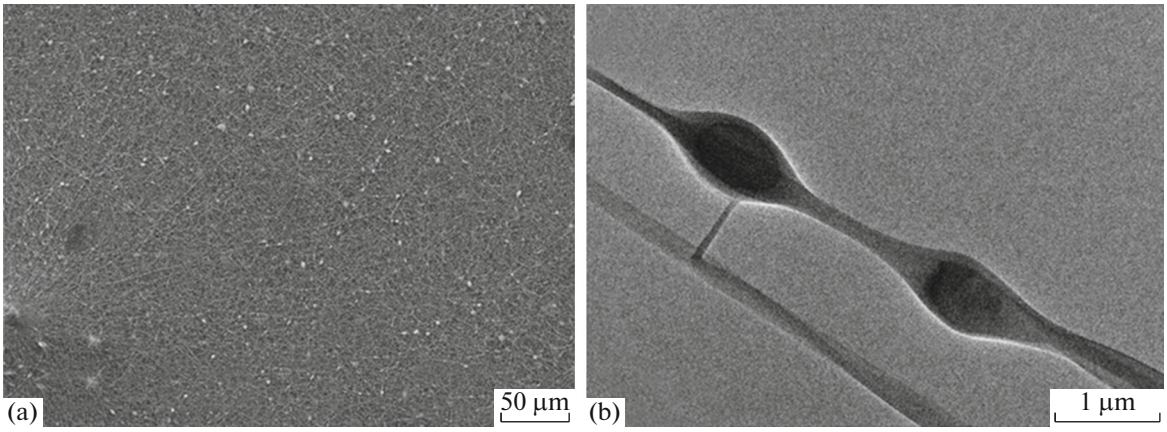


Fig. 2. (a) SEM micrograph and (b) TEM micrograph of PVA composite nanofibers. The flow rate, applied voltage, and distance between needle and collector were set at 0.5 mL/h, 20 kV and 15 cm, respectively. The concentration of PVA was 120 mg/mL.

During electrospinning, the flow rate, applied voltage, and distance between needle and collector were set at 0.5 mL/h, 20 kV, and 15 cm, respectively. The nanofibers produced were deposited on a piece of aluminum foil, on which the electric field was applied. Nanofibers could be formed from PVA with 120 mg/mL concentration and CS microspheres with 15 wt % concentration. From the SEM micrograph of nanofibers showed in the Fig. 2, nanofibers were smooth and CS microspheres fully encapsulated on the surface of fibers, although they have larger diameters than that of fibers. In addition, CS microspheres were coated by nanofibers and transformed to spindles as shown in the TEM micrograph (Fig. 2b).

Processing Parameters of CS-PVA Microspheres Composite Nanofibers

Tests were performed with various concentrations of PVA (100, 120, and 150 mg/mL) and with various concentrations of CS microspheres relative to the PVA (15, 30, and 45 wt %) in order to determine the appropriate amount of polymer to be used. The shape and the size of nanofibers are governed by several parameters such as concentration, viscosity, and surface tension of polymer solution [18], and the results are listed in Table 1.

The initial attempt at electrospinning the suspension liquid of 15 wt % CS microspheres involved three concentrations of PVA (100, 120, and 150 mg/mL). The attempt to electrospin the PVA of 100 mg/mL, resulted in obtaining no nanofibers. Another attempt to electrospin the PVA of 150 mg/mL, also presented a similar problem, which could be owed to that the concentration of PVA was too low to maintain system stability. A large number of microspheres deposited in a short period of time. In these cases, the electrospinning could not be carried out successfully. An explanation proposed regarding the latter has been based on the inability of PVA to form fibers with a high concen-

Table 1. List of property parameters for the polymer solutions

Polymer blend, mg/mL	Viscosity, mPa s	Surface tension, mN/m
100 mg/mL PVA + CS microspheres[a]	7063	44.12
120 mg/mL PVA + CS microspheres[a]	8629	58.35
150 mg/mL PVA+CS microspheres[a]	9350	67.63

[a]The quality of chitosan microspheres was 15 wt %.

tration more than 150 mg/mL at the operation of 20 kV. However, nanofibers could be formed from the PVA of 120 mg/mL with CS microspheres of 15 wt %. The result was shown in the Fig. 3 from A1 to A3. Nanofibers in the picture were smooth and the average size was 0.31 μm. CS microspheres were fully encapsulated by the surface of fibers, although they have lager diameters than fibers. In addition, CS microspheres were transformed to spindles because of the high voltage. Other researchers have also reported similar results. Qi et al. observed in a similar study using electrospinning that a higher voltage resulted in spindle-shaped CS microspheres in nanofibers [19]. Therefore, the 120 mg/mL PVA solution was used in all subsequent experiments.

Under the same electrospinning conditions, different concentrations of CS microspheres relative to the PVA were studied. On the one hand, nanofibers electrospun from the nozzle under similar processing conditions had the same size distribution. On the other hand, the nanofibers appeared to be different when the concentration of the CS microspheres increased. Figure 4 showed that the nanofibers were fractured. Once the concentration of the CS microspheres

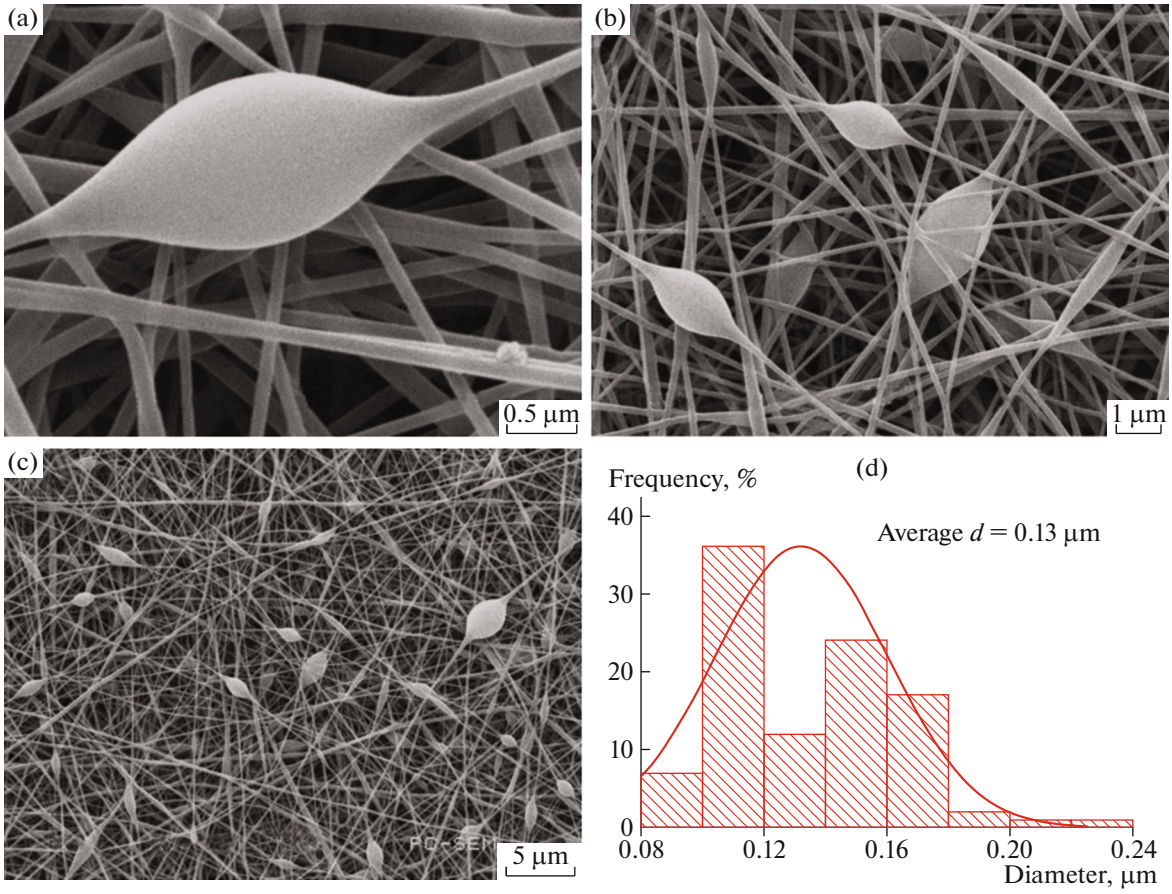


Fig. 3. (Color online) (a–c) SEM micrographs and (d) microsphere size distribution images of nanofibers produced with 120 mg/mL PVA-1 wt % CS microspheres. The flow rate, applied voltage, and distance between needle and collector were set at 0.5 mL/h,20 kV, and 15cm, respectively.

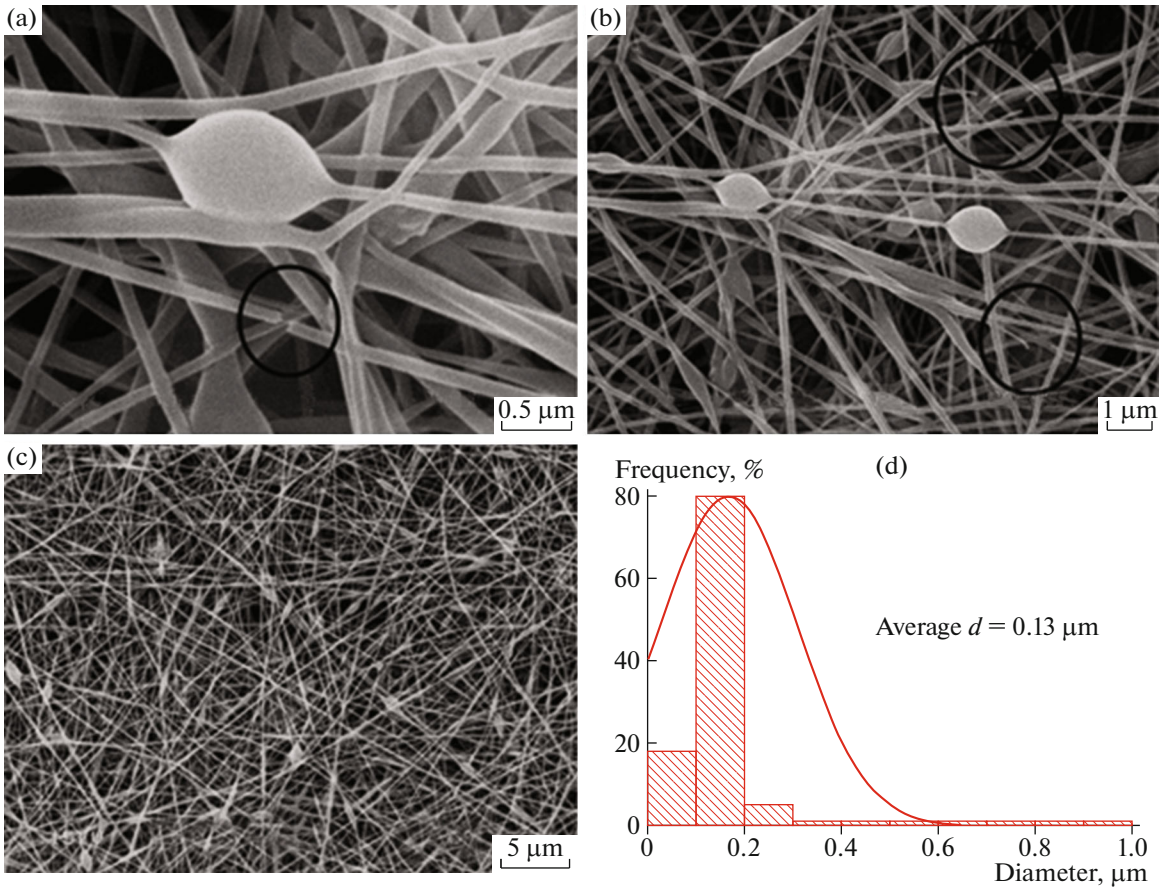


Fig. 4. (Color online) (a–c) SEM micrographs and (d) microsphere size distribution images of nanofibers produced by a solution with 120 mg/mL PVA-30 wt % CS microspheres. The flow rate, applied voltage, and distance between needle and collector were set at 0.5 mL/h, 20 kV and 15 cm, respectively.

increased to 45 wt %, no nanofibers could be produced. The cause of this phenomenon might be that the increase of CS microspheres made the solution difficult to electrospinning. In other words, CS microspheres blocked the formation of nanofibers above a certain content. According to the Table 1, the viscosity of 120 mg/mL PVA solution was 8055 mPa s. The viscosity value of the solution was near to the viscosity of 120 mg/mL PVA solution. CS microspheres have little impact on the solution viscosity. Nanofiber formation in electrospinning is governed by self-assembly processes induced by electric charge [20]. At the tip of the nozzle, PVA is deformed and stretches into nanofibers under the action of a Coulomb force. On the contrary, CS microspheres can't be stretched to nanofibers in this case. At this point, CS microspheres in solution obstruct the formation of continuous nanofibers. Without CS microspheres in solution, PVA nanofibers produced by pure PVA solution were easily and continually formed by electrospinning. When the concentration of CS microspheres (15 wt %) was low, PVA could connect and encapsulate the CS microspheres to form continuous nanofibers because of the high viscosity of the electrospinning solution. Nonetheless, with the increase of CS microspheres, the obstruction to continuous nanofiber formation from CS microspheres would be greater, and the phenomenon of fracture appeared in nanofibers (the red circle in Fig. 4). Once the obstruction from CS microspheres increased to a certain level (the concentration of the CS microspheres reached 45 wt %), no nanofibers could be formed.

X-ray Diffraction

XRD studies of CS powder, PVA powder, CS microspheres, PVA nanofibers, PVA-CS blend nanofibers, PVA-CS microspheres nanofibers and aluminum foil were also investigated in the current study (Fig. 5). There are two peaks in the patterns of CS powder and PVA powder. In the patterns of CS powder, the characteristic peaks were 13.27° and 22.75°, which assigned to crystal forms I and crystal forms II of chitosan [21]. This result indicated that chitosan undergoes two crystallization structures. In the patterns of PVA powder, the characteristic peaks were 14.16° and 22.55°. However, the characteristic peaks of CS or PVA powder were not observed in the diffrac-

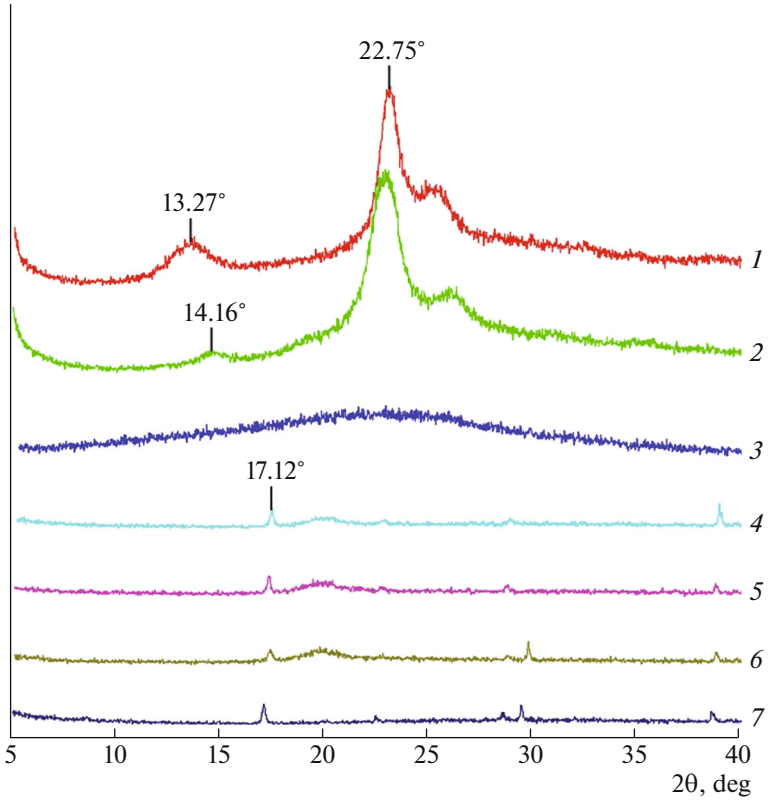


Fig. 5. (Color online) XRD patterns of (*1*) CS powder, (*2*) PVA powder, (*3*) CS microspheres, (*4*) PVA nanofiber, (*5*) PVA-CS blended nanofibers, (*6*) PVA-CS microspheres nanofibers, and (*7*) aluminum foil.

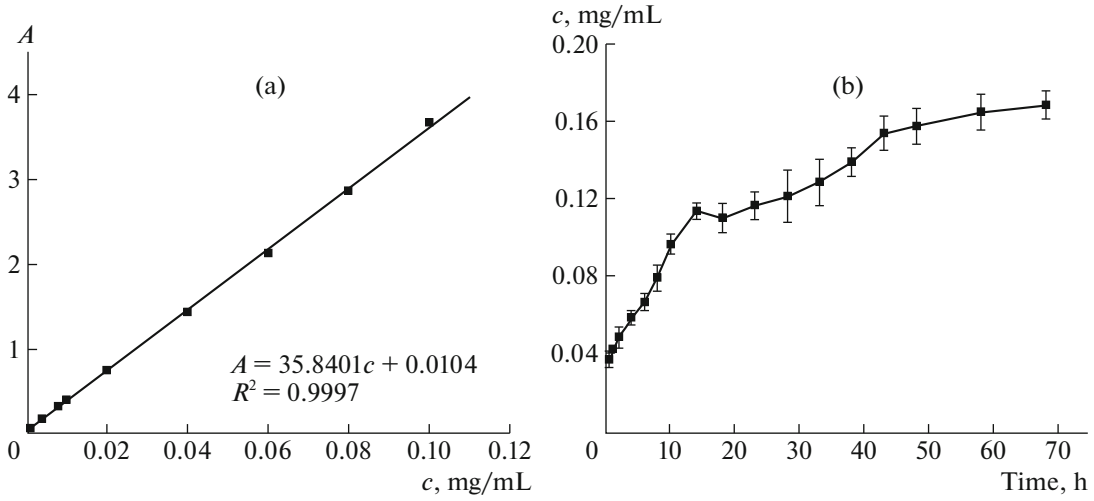


Fig. 6. (a) Standard absorbance concentration curve of CA4: from 0.001 to 0.1 mg/mL and (b) the concentration curve of CA4 in PBS.

tograms of CS microspheres, PVA nanofibers, PVA-CS blend nanofibers, and PVA-CS microspheres nanofibers.

Drug Load and Encapsulation Efficiency

CA4 is poorly soluble in water. The solubility of CA4 in water was only 11.8 ± 1.2 μg/mL [21]. Because of low solubility, measurement errors would be high if the standard absorbance concentration curve of CA4 in phosphate buffered saline (PBS) had been determined directly. In order to improve the solubility of CA4, a surfactant was added to the PBS. The result showed that the solubility of CA4 in PBS increased to 168 μg/mL when the sodium dodecyl sulfate (SDS) was added to PBS. The amount of SDS was 0.005 mg/mL.

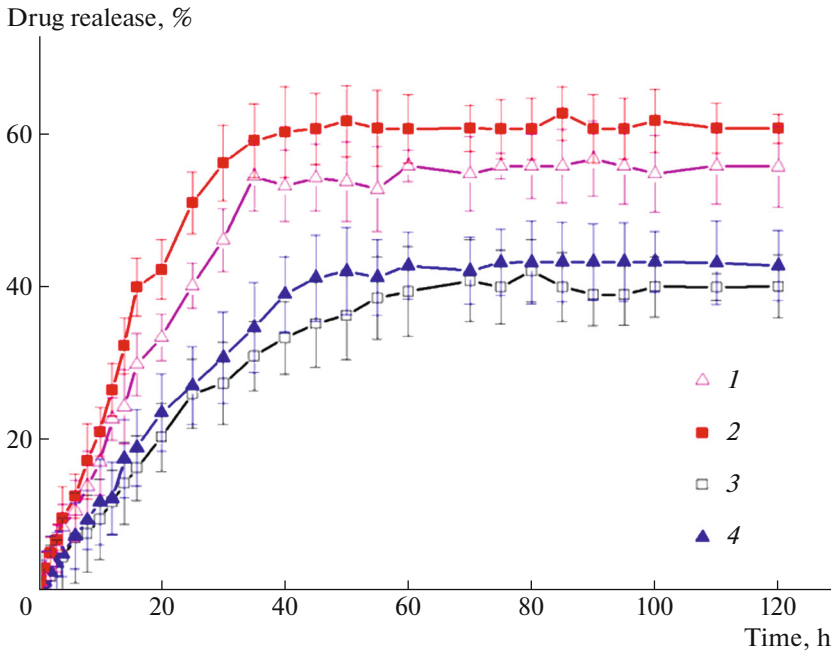


Fig. 7. (Color online) The drug release curve of nanofibers [11] concentration versus time kinetic plot: nanofibers produced by using (*1*), (*3*) 120 mg/mL PVA-15 wt % CS microspheres and (*2*), (*4*) 120 mg/mL PVA-30 wt % CS microspheres; (*3*) and (*4*) were cross-linked by GA steam for 48 h, respectively.

Then, different concentrations of CA4 solution in PBS (with the addition of 0.005 mg/mL of SDS) were prepared. Finally, the standard absorbance concentration curve of CA4 was $A = 35.8401c + 0.0104$, $R^2 = 0.9997$ (Fig. 6).

In this experiment, the drug loading and encapsulation efficiency of microspheres prepared with 50 mg/mL of CS and 15 wt % were 7.54 and 51.00% respectively. The CS microspheres were produced from composite solution. The amounts of added CS-CA4 microspheres in PVA were 15 and 30 wt %, respectively. According to the drug loading and encapsulation efficiency of CS-CA4 microspheres, the amounts of added CA4 in 120 cmg/mL of PVA were 1.36 cm g/mL and 2.72 mg/mL. The results were illustrated in Table 2. The results showed that the drug loading and encapsulation efficiency of PVA-CS microspheres composite nanofibers were 0.7 and 57.4% when the quantity of CS-CA4 microspheres was 15 wt %. In addition, when the quantity of CS-CA4 microspheres was 30 wt %, the drug loading and encapsulation efficiency of PVA-CS microspheres composite nanofibers were 1.1 and 47.2%, respectively. This set of data indicated that the drug loading of composite nanofibers was directly proportional to the quantity of CS-CA4 microspheres. However, the encapsulation efficiency of composite nanofibers produced with the quantity of CS-CA4 microspheres increased.

In vitro Release of CA4

The measurements for CA4 releases were carried out in the PBS (with the addition of 0.005 mg/mL SDS) which functions as a simulated in vivo environment. The release profiles of CA4 from different nanofibers as a function of time are shown in Fig. 7. From the two release curves *1* and *2*, it can be observed that the slow release performances were different when the added amounts of CS-CA4 microspheres were not the same. The nanofibers released around 40% of initially loaded CA4 during the first 40 h when the added

Table 2. Drug load and encapsulation efficiency of PVA-CS microspheres composite nanofibers[a]

PVA, mg/mL	$C^{b}_{\text{CS microspheres}}$, wt %	C_{CA4}, mg/mL	Drug loading, %	Encapsulation efficiency, %
120	15	1.36	0.7	57.4
120	30	2.72	1.1	47.2

[a]The flow rate, applied voltage and distance between needle and collector were set at 0.5 mL/h, 20 kV and 15 cm, respectively.
[b]Relative to the quantity of the PVA.

Table 3. The outcome of statistically significant difference between the OD of experimental group[a] and the OD of negative control group

	24 h	48 h	72 h
P (PVA-15 wt % CS microspheres)[b]	0.09	0.39	0.02
P (PVA-30 wt % CS microspheres)[b]	0.08	0.19	0.46

[a]The flow rate, applied voltage and distance between needle and collector were set at 0.5 mL/h, 20 kV and 15 cm, respectively.
[b]Relative to the quantity of the PVA.

amount of CS-CA4 microspheres was 15 wt %. In addition, only 55% of CA4 was released within 120 h. The nanofibers released 57% of initially loaded CA4 during the first 40 h when the added amount of CS-CA4 microspheres was 30 wt %. After 120 h, the nanofibers released around 60% of CA4.The cause of the phenomenon might be the differences between two kinds of nanofibers. The first kind of nanofibers was continuous. The other kind of nanofibers had a fault in the middle. The nanofibers were cross-linked by glutaraldehyde (GA) steam and the release performance of nanofibers improved clearly. The result is shown in the drug release curves *3* and *4*. The cross-linked nanofibers which were prepared by 15 wt % CS-CA4 microspheres released only 33.1% of CA4 in 40 h. The other cross-linked nanofibers which were prepared by 30 wt % CS-CA4 microspheres released only 38.8% of CA4 in 40 h.

In vitro Cytotoxicity

In order to explore the biological properties of PVA-CS microspheres composite nanofibers, the MTT toxicity test was performed. In the MTT toxicity test, the mouse fibroblast cells (L-929) were used as the model cell. They were soaked in leaching solution for 24, 48, and 72 h respectively. Then, we measured the light absorption value, the optical density (OD), by the enzyme-linked immune detector. The value of OD reflected the activity of cultured cells in material leaching solution. The higher the value, the less toxic were the nanofibers which reflected the biological adaptability of the test materials. Finally, the values of OD were converted to the values of p by the method of a t-test. The confidence level: $a = 0.05$. There is no statistically significant difference between the OD of the experimental group and the OD of the negative control group when the $p > 0.05$.

Experiments could be divided into two groups: experimental group and control group, respectively. In the experiment group, the cultured cells were cultivated by the material leaching solution composed of PVA-CS microspheres composite nanofibers. The control group was composed of a negative control and a positive control. Moreover, there was no material leaching solution in the positive control and the medium in the negative control had a concentration of phenol of 0.64%. Experiment results are as follows: at 24 h, the values of OD (1.00, 1.02) from both experimental groups were higher than the value of OD (0.97) from the negative control in Fig.8.The results indicated that 120 mg/mL PVA-15 wt % CS microspheres composite nanofibers and 120 mg/mL PVA-30 wt % CS microspheres composite nanofibers were non-toxic. At 48 h, the value of OD from 120 mg/mL PVA-30 wt % CS microspheres composite nanofibers (1.12) was higher than the value of OD from negative control (1.10). The result indicated that the PVA-30 wt % CS

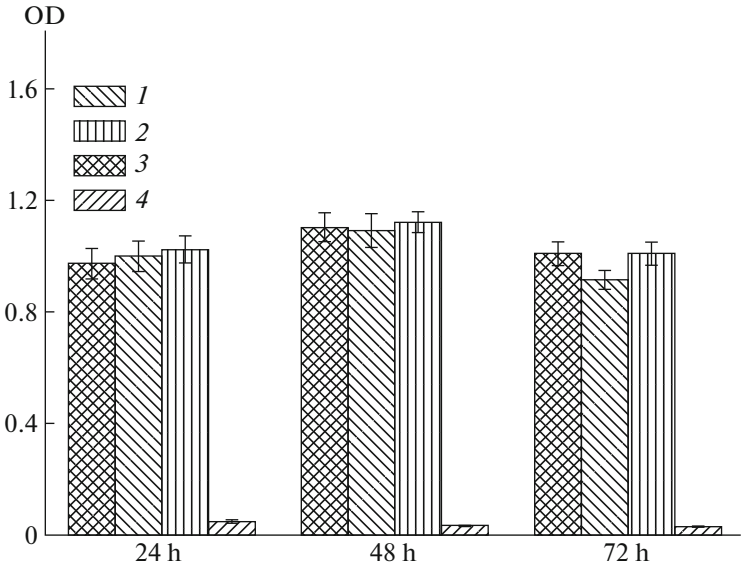


Fig. 8. Cytotoxicity test of (*1*) 120 mg/mL PVA-15 wt % CS microspheres composite nanofiber and (*2*) 120 mg/mL PVA-30 wt % CS microspheres composite nanofiber. (*3*) Negative control, (*4*) positive control.

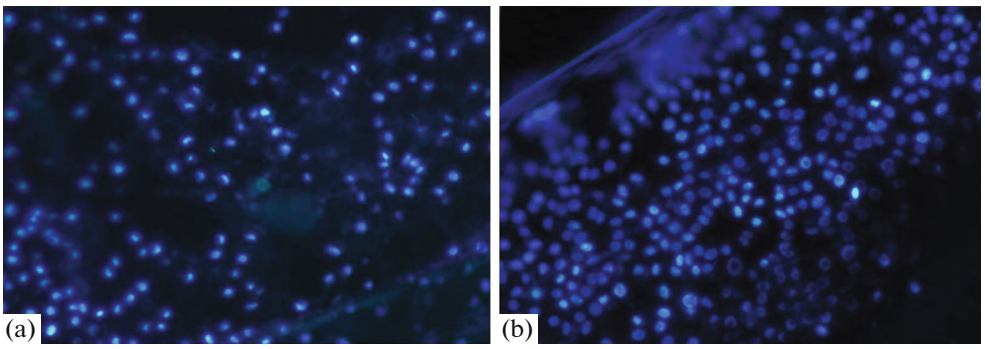


Fig. 9. (Color online) The fluorescence microscopy images of (a) PVA-15 wt % CS microspheres composite nanofibers and (b) PVA-30 wt % CS microspheres composite nanofibers after cell culture for 48 h.

microspheres composite nanofibers were non-toxic. The value of OD from 120 mg/mL PVA-15wt % CS microspheres composite nanofibers (1.09) was a little lower than the value of OD negative control (1.10). However, the value of p from 120 mg/mL PVA-15 wt % CS microspheres composite nanofibers was 0.19 as shown in Table 3. The value was greater than 0.05. This value indicated that there was no significant difference between the value of OD from 120 mg/mL PVA-15 wt % CS microspheres composite nanofibers and the value of OD from the negative control. This suggested that the 120 mg/mL PVA-15 wt % CS microspheres composite nanofibers were non-toxic. At 72 h, the value of OD from 120 mg/mL PVA-30 wt % CS microspheres composite nanofibers (1.1) was higher than the value of OD from negative control (1.01). The PVA-30 wt % CS microspheres composite nanofibers were non-toxic at this time. The value of OD from 120 mg/mL PVA-15 wt % CS microspheres composite nanofibers (0.92) was a little lower than the value of OD negative control. The value of p from 120 mg/mL PVA-15 wt % CS microspheres composite nanofibers was 0.02 as shown in Table 3. The value was lower than 0.05. However, the value of cell activity can still reach 90.8% of the negative control value. It also suggested that 120 mg/mL PVA-15 wt % CS microspheres composite nanofibers were non-toxic. The results show that PVA-CS microspheres composite nanofibers can be used as a biological tissue engineering material.

Cell Apoptosis Analysis

Figure 9 is the fluorescence microscopic image in which fibroblasts cells were cultivated in PVA-CS microspheres composite nanofibers for 48 h. fibroblasts cells attached on the surface of nanofibers. PVA-CS microspheres composite nanofibers had good biocompatibility and were non-toxic. They could promote the adhesion and proliferation on the surface of the nanofibers. The results indicated that PVA-CS microspheres composite nanofibers could be used as a scaffold for tissue engineering.

CONCLUSIONS

Poly(vinyl alcohol)-chitosan microspheres composite nanofibers were successfully obtained by electrospinning technique using a homogeneous suspension liquid. The suspension liquid of 120 mg/mL PVA and 15 wt % chitosan microspheres can produce continuous nanofibers with average size at 0.31 μm. CS microspheres were not exposed on the surface of the fibers. With an increase in the concentration of CS microspheres, the phenomenon of fracture appeared in nanofibers. In the XRD studies, the characteristic peaks of CS or PVA were not observed in the diffractograms after the CS and PVA were processed by high voltage electrostatic technique. The drug loading of composite nanofibers was directly proportional to the quantity of CS-CA4 microspheres. However, the encapsulation efficiency of composite nanofibers produced with the quantity of CS-CA4 microspheres increased. Moreover, the target product of slow release effect is remarkable. Nanofibers were non-toxic and fibroblasts cells attached to the surface of nanofibers.

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