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Biocompatible core–shell electrospun nanofibers as potential application for chemotherapy against ovary cancer

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Polyvinyl alcohol/chitosan (PVA/CS) core–shell nanofibers are successfully fabricated by a simple coaxial electrospinning method, in which PVA forms the core layer and CS forms the shell layer. With the change of the feed ratio between PVA and CS, the surface morphology and the microstructures of the nanofibers are largely changed. The as-prepared core–shell fibers can be used as a carrier for doxorubicin (DOX) delivery. FT-IR analysis demonstrates that hydrogen bond between CS and PVA chains forms. The results of in vitro cytotoxicity test indicate that the core–shell fibers are completely biocompatible and the free DOX shows higher cytotoxicity than the DOX loaded nanofibers. The standing PVA/CS core–shell fibers remarkably promote the attachment, proliferation and spreading of human ovary cancer cells (SKOV3). Via observing by confocal laser scanning microscopy (CLSM), the DOX released from the fibers can be delivered into SKOV3 cell nucleus, which is significant for the future tumor therapy. And, the as-prepared fibers exhibit controlled release for loaded DOX via adjusting the feed ratio between PVA and CS, and the DOX loaded nanofibers are quite effective in prohibiting the SKOV3 ovary cells attachment and proliferation, which are potential for chemotherapy of ovary cancer.

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1. Introduction

Electrospinning, as a simple and versatile technique for producing polymer nanostructures with high surface area-to-volume and length-to-diameter ratios, has been paid extensive attention in the past decades [1]. From the tissue engineering point of view, electrospun nanofibers are the most promising material which could be applicable in medicine and pharmacy because almost all the human tissues and organs, such as bone, dentin, collagen, cartilage and skin, have dimensions on this order. Hereby, in recent years, the nanofibers made from electrospinning have been studied for various biomedical applications, including drug delivery, [2,3] tissue engineering [4–6] and wound dressing [7,8], owing to their interconnected, large surface areas and three-dimensional porous structures.

While as a development of electrospinning method, coaxial electrospinning has received particular attention during the last few years. Various drug and bioactive agents such as antibiotics, DNA, proteins and growth factors could be incorporated directly into the core, which can be protected by the shell, and the results presented a sustained release of these agents from the double layer matrix. For example, Zhang et al. studied the release of fluorescein isothiocyanate-conjugated bovine serum albumin (fitcBSA) from the poly(ethylene glycol)/poly (-caprolactone) (PEG/PCL) core–sheath nanofibers, and the initial burst release was pronouncedly alleviated than the PCL/fitcBSA/PEG nanofibers from simply mixing drugs and carrier polymer by electrospinning [9]. Wang et al. incorporated a proangiogenic compound dimethyloxalylglycine (DMOG) into poly(DL-lactic acid)/poly(3-hydroxy butyrate) (PDLLA/PHB) core–shell fibers, and gave a two-stage kinetics of the DMOG release [10]. Maleki et al. prepared a core–shell structrue with tetracycline hydrochloride (TCH) as the core and poly(L-lactide-co-glycolide) (PLGA) or PLA as the shell, which can be used as a prediction tool in determining drug release profile [11]. Mo et al. demonstrated that bone morphogenetic protein 2 (BMP2) and dexamethasone (DEX) could be incorporated into core–shell nanofibers from poly(L-lactide-co-caprolactone) (PLLACL) and collagen, which showed more controlled release of the growth factors compared with the blended electrospun fibers [12]. Although the release works aforementioned have been quite successful, all of the systems require some organic solvents to dissolve the polymers for electrospinning, which are ill-suited for the biomedical purposes since the residual organics in the resulting fibers may do harm to the organism. Therefore, core–shell structures from materials that are biocompatible, non-toxic, and sometimes also biodegradable are highly desirable.

In the present study, two water-soluble polymers, PVA and CS were used as the initial materials for the fabrication of core–shell fibers by the

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coaxial electrospinning method. PVA was chosen as the core material because of its excellent biocompatibility, biodegradability, good mechanical properties and fiber-forming ability [13,14]. CS, derived through the deacetylation of chitin, possessed perfect biological properties [15,16] and it was selected as a shell polymer. In fact, core–shell structures based on water-soluble materials are not suitable carrier for the controlled release of bioactive agents since the materials will swell rapidly under the physiological conditions. To solve this problem, herein, the CS shell was selectively crosslinked by glutaraldehyde (GA) vapor; consequently, the swelling of polymer chains was effectively restricted. In the in vitro release experiment, an anticancer agent DOX was used as the model drug, which was incorporated into the core of fibers, and its release manner can be effectively controlled by changing the feed rate of CS. And, the potential use of these PVA/CS core–shell nanofibers as a scaffolding material for chemotherapy of ovary cancer was evaluated in vitro against SKOV3 ovary cancer cells. The results showed that the DOX loaded nanofibers were good in prohibiting the cell attachment and proliferation. We anticipate that the resulting core–shell nanofibers will have a tremendous potential in biomedical fields.

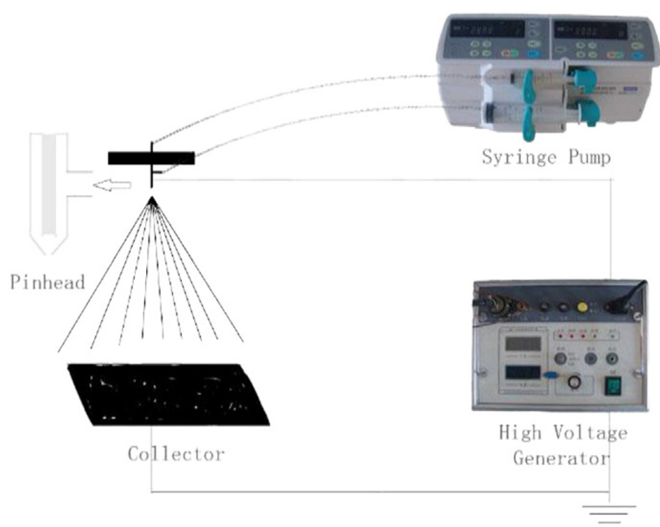
2. Materials and methods

2.1. Materials

Chitosan (CS, degree of deacetylation 0.90, MW 200 kDa) provided by Nantong Shuanglin Biological Product Inc. was refined firstly. PVA (Dp = 1750) was supplied by Changchun Institute of Applied Chemistry Chinese Academy of Science (China). DOX, GA, acrylic acid (AA) and dimethylsulfoxide (DMSO) were used as received.

2.2. Preparation of PVA/CS core–shell nanofibers

Preparation of PVA/CS core–shell nanofibers was conducted according to reference [17]. 0.12 g of purified CS was dissolved in an aqueous AA solution (20 mL, 0.06 g AA), which was used as the shell component of the fibers, and 7 wt.% PVA solution was used as the core material. The setup of electrospinning was shown in Scheme 1. The coaxial spinneret contained two concentrically arranged capillaries. The inner capillary had inner and outer diameters of 0.3 mm and 0.6 mm, respectively, while the outer one had inner and outer diameters of 0.9 mm and 1.15 mm. PVA and CS solutions were loaded in two individual syringe connected to the inner and outer capillary of the coaxial spinneret. The flow rate of solutions in the capillaries was controlled by a pump



Scheme 1. The setup of electrospinning.

with two separate systems. Both capillaries were connected to a high voltage power supply. Aluminum foil, slides or copper net was used as the collector for the fibers. The applied voltage was 18 kV and the distance was 20 cm. The flow rate of PVA was fixed at 1 mL h^{-1}, and the flow rate of CS was set to be 1.0, 1.3 and 1.6 mL h^{-1}, respectively, that is, the feed ratio between PVA and CS was 1:1, 1:1.3 and 1:1.6.

2.3. In vitro drug release experiments

The preparation of DOX loaded core–shell fibers was the same to the process mentioned above, except that a controlled amount of DOX was dispersed into the PVA solution (7 wt.%), which was used as the core component. The obtained drug loaded hybrid nanofibers were crosslinked for 3 min via a GA vapor crosslinking method [18]. After that, the nanofibers taken off from the aluminum foil were immersed into a 2.5 mL pH 7.4 phosphate buffer solution (PBS) at 37 °C. At periodic interval, the release media was withdrawn and another 2.5 mL fresh PBS solution was added. The amount of DOX was calculated by measuring the adsorption at 470 nm (TU-1901 spectrometer, China) and using a calibration curve.

2.4. In vitro cytotoxicity test

Cytotoxicity of PVA/CS core–shell fibers before and after crosslinking, free DOX, and crosslinked DOX loaded core–shell fibers against SKOV3 cells were assessed by MTT assay. Cells were seeded in 96 well plates with a density around 1×10^4 cells/well, and they were allowed to adhere for 24 h. The cells were exposed to a series of doses of samples. After coincubation for 48 h, 20 μL of MTT indicator dye (5 mg mL^{-1} in PBS, pH 7.4) was added to each well and then the cells were incubated for another 4 h at 37 °C in the dark. The medium was withdrawn and 200 μL of DMSO was added in each well to dissolve the crystals of dye. After shaking for 5 min, the data was read on a microplate reader (Bio-Rad, USA). Absorption was measured at a wavelength of 490 nm as reference.

2.5. Internalization of cell

SKOV3 ovary cancer cells were cultured onto slides with crosslinked DOX loaded PVA/CS core–shell fibers in a culture dish in RPMI-1640 medium (Gibco) supplemented with 10 wt.% fetal bovine serum (FBS, Hyclone, Logan, UT), streptomycin at 100 μg mL^{-1}, penicillin at 100 IU mL^{-1} and 4 mM mL^{-1} glutamine at 37 °C in a humidified 5 wt.% CO_2 containing atmosphere. In this experiment, DOX was loaded into PVA core and CS was labeled with FITC.

After 3 days' culture, the slides with cell monolayer were taken out of the medium, washed using ice-cold PBS and distilled water, and fixed using fresh GA 1.25 wt.% for 5 min. Then, the fixative was removed, and fresh GA was added to fix it for another 10 min. The slides were washed with ice-cold PBS and distilled water alternatively for three times, followed by removing the medium and drying in the air. The nuclei of these cells were stained with 5 μg mL^{-1} Hoechst 33258 under light-sealed condition for 30 min. Then, the slides were washed with PBS and distilled water to remove the extra dye molecules. After drying in the air, the slides were added with avoiding decay solvent, occluded and observed under a confocal laser scanning microscopy (TCS sp2 AOBS, Germany).

2.6. Cell culture and adhesion

SKOV3 cells were seeded onto an aluminum foil with crosslinked PVA/CS core–shell nanofibers and DOX loaded PVA/CS core–shell nanofibers. After culture for 1 day, 3 days and 8 days, the samples were rinsed twice with PBS buffer to remove non-adherent cells, and subsequently fixed with 2.5% GA at 4 °C for 2 h. After that, the samples were dehydrated through a series of gradient ethanol solutions of 30%, 50%,

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