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Enhancement of antioxidant and antibacterial properties for tannin acid/chitosan/tripolyphosphate nanoparticles filled electrospinning films: Surface modification of sliver nanoparticles

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ABSTRACT

The tannin acid/chitosan/tripolyphosphate nanoparticles were encapsulated in polyvinyl alcohol (PVA)/poly-acrylic acid (PAA) electrospinning films by electrostatic spinning technology. To optimize the prepared condition, properties and morphology of nanoparticles were characterized by dynamic light scattering (DLS) and transmission electron microscope (TEM). The optimized initial concentration of tannin, chitosan and tripolyphosphate solutions were 1, 1, 0.5 mg/ml, respectively, with adding proportion for 5:5:1. The average diameter of tannin acid/chitosan/tripolyphosphate nanoparticles was ~80 nm. The electrospinning films showed an excellent water-resistant property with 0.5 wt% *N,N′*-Methylenebisacrylamide (MBA). Due to the antioxidant and antibacterial of tannic acid, the films possessed these properties. The antioxidant and antibacterial of these fibers significantly improved after in situ formation of silver nanoparticles (AgNPs). Electrospun films were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectra (FT-IR), X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS).

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1. Introduction

During the last few years, the development of polymeric materials with antimicrobial and antioxidant properties has prompted great interest due to the wide range of potential applications of polymer materials [1,2]. Antimicrobial agents can reduce or inhibit microbial pollution for food, which is a major problem for human health [3]. In addition, there is an increasing demand for materials with antioxidant function, which can reduce the excessive levels of free radicals and extend the shelf life of food products [4]. Therefore, the manufacture of polymeric materials with both antibacterial and antioxidant functions would be greatly momentous.

At present, electrospinning is widely considered as the most efficient technique for producing polymer fibers with diameter on nano or sub-micron scale [5]. Electrospinning films possess high specific surface area and have received much attention because of their potential applications for antibacterial coating, biomedical devices, tissue engineering scaffolds, and absorbent materials, etc [6,7]. Electrospinning films have design flexibility for particular functionalization which can be performed by applying post-treatment methods or during the electrospinning process. For example, functional composite electrospinning films can be produced by incorporation of metal nanoparticles (NPs), such as silver or gold, into electrospun polymeric nanofibers [8,9]. Silver nanoparticles (AgNPs) have attracted considerable attention due to their unique optical, electronic, catalytic and antibacterial properties [10,11]. Numerous approaches were followed to obtain polymer/AgNPs composite electrospinning films. However, in these approaches silver salt precursor was directly added into the polymer solution and the synthesis of AgNPs was carried out through thermal [12], chemical [13] and photoreductive [14] post-treatment of the electrospinning films. So these methods mentioned above are often time-consuming, and mostly require reducing and stabilizing chemicals which are sometimes highly toxic. Therefore, simple and green approaches are essential for the practical applications of electrospinning films incorporating AgNPs. Many kinds of polyphenols from plants are absorbed on electrospinning films for in situ reduction of silver ions, making the

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films functionalization. For instance, silver nanoparticles (AgNPs) were synthesized with grape seed polyphenols as reducing agent in aqueous solution of gelatin [15]. Zou [16] proposed introducing tea polyphenols as the green reductant to synthesize AgNPs through an in situ reduction approach in polyacrylonitrile/dimethyl formamide solution. Tannic acid (TA) is a glucoside of gallic acid polymer with multiple phenolic hydroxyl groups that are found in many plants [17]. This is an attractive molecule known to have antitumor, antibacterial, and antioxidant activity [18], and also be used as a reducing agent. These properties provided the potential utilization of TA in developing active packaging materials [19].

In this present work, AgNPs loaded PVA/PAA nanofibers were synthesized by using TA as the reductant. However, TA is rich in phenolic hydroxyl groups, which is easy to form a strong hydrogen-bonding with the alcoholic hydroxyl groups of PVA, leading to the flocculation of electrospinning solution. Chitosan (CS) is a kind of polysaccharide and has been widely used for preparation of composite materials, which is known to be biocompatible, degradable, and possess antibacterial properties [20–23]. Therefore, we synthesized tannin acid/chitosan nanoparticles (TA/CS-NPs) with tripolyphosphate (TPP) as crosslinking agent to weaken the hydrogen-bonding between TA and PVA and then the TA/CS/TPP-NPs/PVA/PAA mixed solution was electrospun into films. The *N*,*N*′-Methylenebisacrylamide (MBA) was used as a crosslinking agent, competitive to react with the hydroxyl group of PVA and the carboxyl group of PAA. The morphology, structure and water-resistant properties of the electrospinning films was investigated. At the same time, by using the reducing capacity of TA, The TA/CS/TPP-NPs containing electrospinning films were immersed in silver nitrate solution to obtain AgNPs loaded electrospining films. The antibacterial and antioxidant activity of the composite electrospinning films were evaluated.

2. Materials and methods

2.1. Materials

Chitosan (CS, $M_w = 2.0 \times 10^5$ kDa) from shrimp shell with deacetylation of 92% was purchased from Zhejiang Yuhuan Ocean Biochemical Co., China. Tannic acid (TA) and sodium tripolyphosphate (TPP) were purchased from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China). Polyvinyl alcohol (PVA) and N, N'-Methylenebisacrylamide (MBA) were purchased from the Aladdin Chemical Co., China. Polyacrylic acid (PAA) was purchased from Sigma-Aldrich Co., USA. The other reagents were analytical grade purchased from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China). *E.coli* and *S. aureus* were obtained from China Center for Type Cultuer 140 Collection, Wuhan University (Wuhan, China).

2.2. Preparation of TA/CS/TPP-NPs

TA/CS/TPP-NPs was prepared using a modified method that has been reported previously and the optimum preparation condition was studied [24]. CS solution(5 mg/ml) was prepared by dissolving CS in acetic acid solution until the solution was transparent. The concentration of TA solution was 5 mg/ml. CS and TA solution was diluted with pure water to obtain different concentrations at 0.25, 0.5, 1, 2, 3 mg/ml accordingly. TPP was dissolved in pure water at the concentration of 0.5 mg/ml. The TA solution was added slowly to CS solution and the weight ratios of CS to TA were 3:1, 2:1, 1:1, 1:2 and 1:3. Then the TPP solution was added into the mixture. The nanoparticles suspensions were gently stirred for 2 h at room temperature before being subjected to further analysis and applications. The diameter, zeta potential and polydispersity index (PDI) of nanoparticles were measured to evaluate the optimum preparation conditions by DLS. Morphology of nanoparticles was characterized by TEM.

2.3. Fabrication of TA/CS/TPP-NPs electrospun films

TA/CS/TPP-NPs electrospining films were fabricated by using a set of homemade electrospinning setup, which contained a high voltage supply (DW-P303-1ACD8, Tianjin Dongwen Co., China), and a syringe pump (LSP02-1B, Baoding Longer Precision Pump Co., Ltd., China). PVA(800 mg) was dissolved in 9.2 g deionized water at 90 °C overnight to obtain 8% pure PVA solution. Appropriate amount of PAA was added to the PVA solution at room temperature and the final weight ratio of PVA and PAA was 7:3 [25]. Then, different weights of TA/CS/TPP-NPs were added into the PVA/PAA spinning solution to find the optimum electrospinning concentration. Rhodamine B was added into CS solution to obtain fluorescent TA/CS/TPP-NPs, and the fluorescent TA/CS/TPP-NPs solution was spun to study the distribution of TA/CS/TPP-NPs in electrospinning films. At the optimum electrospun conditions, different weights of MBA(0.25–2.0 wt%) were added into the TA/CS/TPP-NPs/PVA/PAA spinning solution to investigate the effect of MBA on the water solubility of electrospining films [26].

2.4. Preparation of Ag/TA/CS/TPP-NPs electrospining films

Synthesis of the Ag/TA/CS/TPP-NPs electrospining films was based on previous study with some modifications [27]. In a typical synthesis, 0.2 g TA/CS/TPP-NPs electrospining film was soaked in 10 ml silver nitrate solution (20 mM) for 3 h. Ag-NPs loaded films were dried at 20 °C for overnight under vacuum prior.

2.5. Characterization

Scanning electron microscopy (SEM) (S-4800, Hitachi Ltd., Japan) was used to characterize the morphology and composition of electrospining films. The fiber diameter was measured by an image analyzer (Adobe Photoshop CS5.0). Fourier transform infrared (FT-IR) spectra were recorded by using a Nicolet 170-SX instrument (Thermo Nicolet Ltd., USA) in the wavenumber range of 4000–400 cm^{-1}. X-ray diffraction (XRD) was carried out using a diffract meter type D/max-rA (Rigaku Co., Japan) with Cu target and Ka radiation ($\lambda = 0.154$ nm). Particle size distributions of TA/CS/TPP-NPs were determined by using dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). X-ray photoelectron spectroscopy (XPS) was conducted on an axis ultra DLD apparatus (Kratos, UK). The distribution of the TA/CS/TPP-NPs and TA/CS/TPP-AgNPs in electrospining films was studied by fluorescence microscope (80i, Nikon instrument Co., Ltd, Japan).

2.6. In vitro antibacterial activity assay

Antibacterial activity of electrospining films was assayed by the inhibition zone test [28]. The Gram-negative *E.coli* and Gram-positive *S.aureus* were selected as representative microorganism and were cultivated in the culture medium in an incubator. The TA/CS/TPP-NPs films and Ag/TA/CS/TPP-NPs film were cut into 6 mm round disks with the hole puncher, and non-NPs-films was used as negative control. One hundred microlitre of overnight cultured *E.coli* and *S.aureus* bacteria levitation liquid ($5.0–10.0 \times 10^5$ cfu/ml) was added onto the 25 ml of agar medium and coated uniformly, respectively. The prepared films were placed on the inoculated plates. Then, the plates were cultured at 37 °C for 24 h in an incubator and the diameters of the inhibition zones were measured by a vernier caliper. All of the experiments were conducted in triplicate with data reported as mean ± standard deviation.

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