International Journal of Biological Macromolecules 91 (2016) 68–74

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Release properties of tannic acid from hydrogen bond driven antioxidative cellulose nanofibrous films

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ARTICLE INFO

Article history:
Received 31 January 2016
Received in revised form 10 May 2016
Accepted 23 May 2016
Available online 24 May 2016

Keywords:
Layer-by-layer assembly
Electrospun nanofibers
Hydrogen bonds
Polyphenolic drug
Antioxidant acitivity

ABSTRACT

Layer-by-layer (LBL) assembled films have been exploited for surface-mediated bioactive compound delivery. Here, an antioxidative hydrogen-bonded multilayer electrospun nanofibrous film was fabricated from tannic acid (TA), acting as a polyphenolic antioxidant, and poly(ethylene glycol) (PEG) via layer-by-layer assembly. It overcame the burst release behavior of nanofibrous carrier, due to the reversible/dynamic nature of hydrogen bond, which was responded to external stimuli. The PEG/TA nanofibrous films disassembled gradually and released TA to the media, when soaked in aqueous solutions. The release rate of TA increased with increasing bilayer number, pH and temperature, but decreased with enhancing ionic strength. The surface morphology of the nanofibrous mats was observed by scanning electron microscopy (SEM). The following antioxidant activity assay revealed that it could scavenge DPPH free radicals and $ABTS^{+\bullet}$ cation radicals, a major biological activity of polyphenols. This technology can be used to fabricate other phenolic-containing slowly releasing antioxidative nanofibrous films.

1. Introduction

Electrospinning is a powerful and straightforward way for fabricating nanofibers with controllable compositions and structures [1,2]. Electrospun nanofibers with high surface area to volume ratio and high porosity have received much attention for their potential applications in the areas of smart packaging, biomedical devices, wound dressing, and drug delivery [3]. To improve the functions for biomedical uses, the surfaces of electropsun nanofibers have been modified with some bioactive molecules or drugs after the electrospinning process via different methods. [4,5]. Among the numerous modified technologies, layer-by-layer (LBL) assembly has been extensively adopted to fabricate multilayer thin films using polymer pairing with complementary functional groups of bioactive molecules. This assembly has its advantages over other methods, such as no limit to the complex structure of substrate, and easy control of film thickness and composition [6]. Through LBL assembly technology, a variety of drugs or bioactive molecules can be immobilized onto the nanofibrous surface without compromising bulk properties. When soaked in designated solutions, these drugs or molecules could release from the thin films via diffusion alone or diffusion with the film skeleton degradation [7].

Tannic acid (TA) is a natural polyphenol with multiple hydroxyl groups on the aromatic rings [8]. TA-based assemblies have recently drawn some attention due to their capability to interact with multiple substrates and their diverse biological properties including antioxidant, antibacterial, astringent and anti-carcinogenic activity [9–12]. In addition, due to TA's high pK_a value of ca. 8.5, its association through hydrogen bonding with some polymers is expected to occur at neutral and acid pH values [13]. Recently, it has been incorporated in hydrogen-bonded LBL films at physiologic pH [13]. To date, little attention was paid to the fabrication of TA hydrogen-bonded multilayer electrospun nanofibrous films and the release properties of TA from hydrogen-bonded multilayer electrospun nanofibrous films at various conditions.

In current work, we are fabricating multilayer nanofibrous mats using TA, a polyphenolic drug, and poly(ethylene glycol) (PEG) via layer-by-layer assembly driven by hydrogen bonds. Due to the reversible/dynamic nature of hydrogen bonding, the multilayer thin films can gradually disassemble and release TA into the media under suitable conditions. To test this hypothesis, the release profiles are compared with the multilayer films deposited on a flat surface. The antioxidant assays such as DPPH radicals and $ABTS^{+\bullet}$ cation radicals scavenging assays are further used to exam-

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http://dx.doi.org/10.1016/j.ijbiomac.2016.05.084
0141-8130/

ine whether the mats could retain TA's antioxidant activity, a major biological activity of polyphenols.

2. Materials and experimental

Cellulose acetate (CA, *Mn* = 30000) was purchased from Sigma–Aldrich Co., (Los Angeles, USA). Poly(ethylene glycol) (PEG, *Mn* = 400, 1000, 2000, 4000, 6000) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Tannic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) was obtained from Aladdin Chemistry Co. Ltd. (Shanghai, China). Other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). NKA-9 macroporous adsorption resin was purchased from Tianjin Nankai Hecheng S&T Co. Ltd (Tianjin, China). It was pretreated according to the user's manual before use. All aqueous solutions were prepared using ultrapure water with a resistance of 18.2 MΩ cm^{-1} through a Millipore (Millipore, Milford, MA, USA) Milli-Q water purification system.

3. Experimental

3.1. Preparation of cellulose nanofibers

Nanofibrous CA mats were fabricated according to Ding's method with some modifications [14]. The CA solution was prepared in acetone and *N*,*N*-dimethyl acetamide (DMAc) solution with an acetone/DMAc weight ratio of 2:1. Then it was transferred into a plastic syringe, which was driven by a syringe pump (LSP02-1B, Baoding Longer Precision Pump Co., Ltd., China). The positive electrode of a high voltage power supply (DW-P303-1ACD8, Tianjin Dongwen Co., China) was clamped to the metal needle tip of the syringe. A grounded cylindrical layer was used as a collector which rotated at 50 rpm. The applied voltage was 17 KV and the tip-to-collector distance was 20 cm. The ambient temperature and relative humidity were maintained at 25 °C and 45%, respectively. The prepared fibrous mats were dried at 353 K in vacuum for 24 h to remove the trace solvent. The hydrolysis of CA mats were performed in alkaline aqueous solution at an ambient temperature for 7 days following the previous reported procedures [15,16].

3.2. Formation of nanocomposite films on template nanofibers

The pH value of PEG and TA solution (1 mg/mL) was adjusted to 3.0 using 0.1 M hydrogen chloride solution. To fabricate the LBL films, the substrates were immersed in 50 mL of PEG and TA alternately, each for 10 min. Then, the solution was suction-filtered through the nanofibrous mats. Following each deposition step, the mats were washed with 50 mL of hydrogen chloride solution (pH = 3.0) [17]. This cycle was repeated until the desired bilayer number was reached. The resultant mats were denoted as "$(PEG/TA)_n$", which means the mats is fabricated from PEG and TA with a bilayer number of n. The LBL films coated nanofibrous mats were dried at room temperature under vacuum prior to further characterization.

3.3. Characterization of nanofibrous mats

The morphology characterization of the composite membranes was performed using scanning electron microscopy (SEM) (S-4800, Hitachi Ltd., Japan). Fourier transform infrared (FT-IR) spectra were acquired on a Nicolet170-SX instrument (Thermo Nicolet Ltd., USA) in the wavenumber range of 4000–400 cm^{-1}.

3.4. Release of TA

To evaluate the release kinetics of TA, 6 mg of PEG/TA nanofibrous mats ($(PEG/TA)_{15}$, unless otherwise specified) were immersed in 30 mL of phosphate buffer (usually 0.2 M, pH 7.4, unless otherwise specified). The temperature was controlled with a refrigerated circulator. At appropriate intervals, the release media were removed, and the same volume of fresh media with the same temperature was added. Concentration of TA in the release media was determined using UV/vis spectroscopy at a wavelength of 276 nm.

3.5. In vitro *antioxidant activity assay*

3.5.1. DPPH radical scavenging activity

The antioxidant activity of nanofibrous mats was measured according to the published DPPH scavenging method with minor modification [17]. Briefly, scavenging activity assay was carried out by recording the absorbance of DPPH solution (100 mM) at 517 nm in the presence of the nanofibrous mats at room temperature with a UV–vis spectrophotometer. The free radical scavenging potency of the nanofibrous mats were expressed as the percentage of DPPH that was decreased in comparison with that of the control condition after a certain period of preservation time in the dark. Moreover, the $DPPH^{\bullet}$-loaded resin was then soaked in ethanol. A $(PEG/TA)_{15}$ nanofibrous mat (Φ = 6 mm) was placed under the resin. The decoloration of the resin with time was recorded with a digital camera.

3.5.2. ABTS radical scavenging activity

ABTS radical scavenging activity was assayed as per the method of Wu jiangping with a slight modification [18]. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted with phosphate buffer in order to obtain an absorbance of about 1.30 units at 734 nm using a microplate reader. The fresh ABTS solution was prepared for each assay. Nanofibrous mats (1 mg) were put in 5 mL of ABTS solution and the mixture was left at room temperature for a certain period of time in the dark. The absorbance was then measured at 734 nm using a spectrophotometer. Sample blank was prepared in the same manner except that phosphate buffer was used instead of ABTS solution. In addition, the $ABTS^{+\bullet}$-loaded resin was then soaked in pH 7.4 phosphate buffer. Four pieces of $(PEG/TA)_{15}$ nanofibrous mats (Φ = 6 mm) were placed under the resin. The decoloration of the resin with time was recorded with a digital camera.

4. Results and discussion

4.1. Fabrication of PEG/TA nanofibrous mats

Layer-by-layer (LBL) assembled technology has become a powerful tool to fabricate multilayer films using polymer that pairs with complementary functional groups of bioactive molecules driven by various interactions between assembling components [6]. Here, the multilayer nanofibrous films were constructed by hydrogen bonding between TA and PEG, whose structures were shown in Scheme 1. Firstly, to evaluate whether there is strong interaction between TA and PEG, TA solution (1 mg/mL) was added into the PEG solution with different molecular weight. It was observed that the transparent solution became cloudy immediately. Additionally, the turbidity of the mixed solution enhanced with the increase of the molecular weight of PEG. From the TEM image in Fig. 1, it was observed that these two components (TA and PEG, *Mn* = 6000) formed nanoparticles with well-defined spherical shape. The result