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Dual responsive zein hydrogel membrane with selective protein adsorption and sustained release property

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ABSTRACT

Drug-loaded hydrogels have been paid increasing attentions in biomedical fields. As a sort of natural plant protein, zein generally cannot form hydrogel with high water retention because of its predominant hydrophobicity, which will limit its application as biomaterial. In this paper, zein electrospun fibrous membranes (ZEFM) are fabricated through a chemical modification of zein using citric acid and acetic anhydride. The resulting ZEFM can be totally soluble in neutral phosphate buffer solution. After being crosslinked by sodium hexametaphosphate, the ZEFM can form a hydrogel membrane and displays stimuli-responsive behavior towards pH and ionic strength. The hydrogel membrane exhibits better protein adsorption, selectivity and sustained release profile for positively-charged proteins such as cytochrome *C*, compared with those unmodified ones, and also shows fast biodegradation behavior and qualified cytotoxicity, which all make it favourable for biomedical use.

1. Introduction

Recently, hydrogels have become more and more attractive in tissue engineering and regenerative medicine because of their high water retention and similar viscoelasticity with living tissues [1,2]. Depending on the crosslinked network structure, they can also deliver therapeutic drugs to accelerate the repairment of tissues and organs [3,4]. To construct such hydrogel biomaterials, natural polymers are more acceptable based on their superior biocompatibility and degradability in physiological conditions [5,6]. Many hydrogels originated from natural polysaccharides (chitosan [7–9], alginate [10–13], hyaluronic acid [14,15], etc.) and animal proteins (collagen [16,17], gelatin [18–20], fibrinogen [21], etc.) have been reported and even widely used in the clinic. Compared with animal proteins, natural plant proteins will be benefit because of the low cost, extensive sources and less immunogenicity [3–6]. Therefore, it is meaningful to develop plant protein based hydrogels for biomedical application.

Zein is a natural plant protein extracted from corn. As a sort of prolamin, it is rich in nonpolar amino acids (~53%), but deficient in basic and acidic ones (~30%), resulting in its high hydrophobicity and water insoluble behavior [22–25]. Zein has been widely used in food packaging and drug delivery systems [26,27], and zein films are also used as functional coating for various biomedical materials [28,29]. A main disadvantage of pure zein films/membranes is their tendency to shrink or even collapse in water [30], which is not favourable for some film-based biomaterials, e.g., wound dressing. To improve the water-resistance, and simultaneously to enhance the plasticity and mechanical strength of the zein films, many physical treatments [31–34] and chemical modifications [35–39] have been adopted. Nevertheless, to our best knowledge, a practical zein hydrogel membrane with high water retention has not yet been fabricated until now.

In the present work, we modified zein by citric acid (CA) together with acetic anhydride (AA) under a non-aqueous acetic acid (AcOH) environment, and a saline-soluble zein electrospun fibrous membrane (ZEFM) was prepared after electrospinning (as illustrated in Scheme 1). The eco-friendly reagents CA and AA were used to regulate the hydrophilicity/hydrophobicity of zein, and the electrospinning method was used to form membranes by removing the solvent rapidly and thoroughly. Then after further being crosslinked with a polycyclic phosphate, sodium hexametaphosphate (SHMP), a water-swellable ZEFM was prepared, which could be swollen as a hydrogel in both pure water and phosphate buffer solution (PBS) and exhibited pH- and ionic strength-responsive behavior. The whole reaction would be carried out under mild and green conditions, preventing the utilization of toxic solvents and some cytotoxic or costly crosslinking agents (such as glutaraldehyde, formaldehyde, and carbodiimide, etc.). The modification degree and the primary molecular structure of zein, the microstructure, water contact angle, swelling ratios, protein adsorption and release

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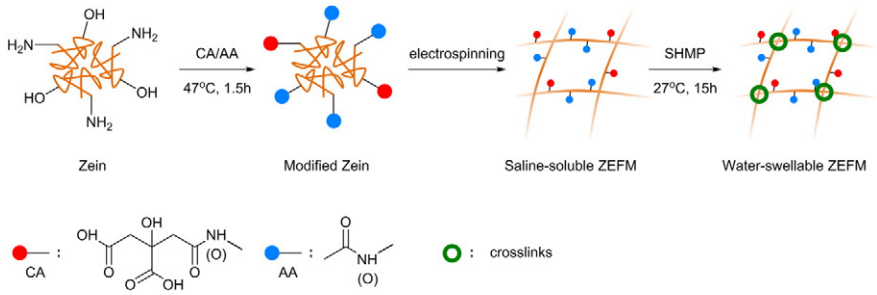

Scheme 1. Schematic of the preparation process of saline-soluble and water-swellable ZEFM.

profiles, degradation profiles, and the cytotoxicity of the modified ZEFMs were all determined and compared with the unmodified ones.

2. Experimental section

2.1. Materials

Zein (food grade, used without further purification, Mn = 20–22 kDa, protein content > 98.5%, fat < 0.5%, water < 1%, ash content < 0.2%) was purchased from Hufeng Chemical Industry Co. Ltd. (Shanghai, China). Glacial acetic acid (AcOH, A.R.) was purchased from Tianjin Chemical Reagent No. 1 Plant (Tianjin, China). CA (A.R.), SHMP (A.R.) and phenol were purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). AA was purchased from Tianjin Jinfeng Chemical Reagent Factory (Tianjin, China). EDC (A.R.) and N-hydroxysuccinimide (NHS, A.R.) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA, Mw = 68 kDa) was purchased from Jianyang Bio-technology Co. Ltd. (Guangzhou, China). Cytochrome *C* (Cyt *C*) (>95%, Mw = 12 kDa) was purchased from Sigma-Aldrich Chemical Co. Ltd. (St. Louis, MO, USA). The mouse embryonic fibroblasts cell line (ATCC CRL-1658) was obtained from the American Type Culture Collection (Rockville, MD). Dulbecco's modified Eagle's medium (DEME) was purchased from Lifei Bio-technology Co. Ltd. (Shanghai, China). Fetal calf serum and double-antibody were obtained from Life Technologies Co. Ltd. (Grand Island, NK, USA). Bicinchoninic acid (BCA) kits and cell Counting Kit-8 (CCK8, 500T) were both obtained from Bestbio (Shanghai, China). PBS with different pH values and concentrations were prepared freshly before use. All other chemicals were of analytical grade, and obtained from commercial companies.

2.2. Modification of zein with CA/AA and the electrospinning

CA was dried at 80 °C for 4 h and dehydrated at 110 °C for 1 h. A certain amount of anhydrous CA was dissolved in AA by heating to get a homogenous solution at a humidity <40%. 1 g of zein powder was dried at 50 °C in advance, dissolved in dehydrated AcOH at a humidity <40%, and kept stirring at room temperature for 40 min to get a completely homogeneous zein solution. Then the as-prepared CA/AA solution was added dropwise into the zein solution. The mixture continued to be stirred at a certain temperature (37, 42, 47 °C) for 1.5 h before electrospinning into membranes. The final concentration of zein and CA in the mixture was in a range of 15–27 wt.% and 0.9–5.2 wt.%, respectively, and the concentration of AA was fixed as 5.5 wt.%. The corresponding membranes were named as ZM-1–ZM-9 and ZM-13 (Table 1). Simultaneously, blank samples (ZM-0, ZM-12) were also prepared by using 27 wt.% or 15 wt.% zein-AcOH solution. And the sample only containing CA (ZM-10) or AA (ZM-11) was prepared as a comparison, by using 15 wt.% zein-AcOH solution mixed with 5.2 wt.% CA (dispersed in AcOH solution) or 5.5 wt.% AA under the same conditions. The ZMs with different components were listed in Table 1, which were all obtained through electrospinning under the same conditions as below.

The apparatus used for electrospinning was homemade and described in our previous article [40]. The electrospinning processes were carried out at 22 ± 4 °C with humidity of 55 ± 3%. The applied voltage was fixed as 27.0 kV and the flow rate was kept at 0.8 mL/h. The nanofibers were deposited on a 15 cm × 15 cm metal board (covered by an aluminum foil) at a distance of 10–15 cm to form fibrous membranes. The as-electrospun membrane was then removed from the board and dried at 50 °C for 48 h to get rid of the residual solvent.

2.3. Crosslinking of ZMs with SHMP

The dried CA/AA-modified and unmodified ZMs in 2.2 (all around 1–1.5 cm × 1–1.5 cm) were immersed in a saturated SHMP aqueous solution at 27 ± 1 °C over 15 h, and gradually adjusted the acidity to pH > 13. The obtained samples (named as C-ZM-serial number accordingly) were firstly washed by 0.2 mol/L PBS and then by 1.56×10^{-3} mol/L PBS (or pure water towards the C-ZM-0) for several times to remove the reaction residuals, and dried under 50 °C to get the dry membranes.

2.4. Characterization

2.4.1. Viscosity determination

The viscosity of the pre-electrospinning solution of ZEFM versus shear rate was determined using a rheometer (Kinexus pro+, Malvern, UK) with the shear rate in the range of 0.1–100 s^{-1} at 25 °C.

2.4.2. X-ray photoelectron spectroscopy (XPS)

The ZMs (ZM-0 and ZM-6) were immersed in pure water for 3 days by constantly changing water, and dried at 50 °C before XPS determination. The XPS determination was carried out under an X-ray photoelectron spectrometer (ESCALAB 250, Thermo Fisher Scientific, USA) using Mono AlK_α radiation (1486.6 eV, 15 kV 150 W). The vacuum degree in the chamber was in the range of 10^{-9} mbar. The energy scale was calibrated by using the surface contaminated C1s (284.8 eV). The constant pass energy and energy step size was 100 eV and 1 eV/step for full-scan

Table 1
ZMs with different components.

Samples	Contents			
	Zein (wt.%)	CA (wt.%)	AA (wt.%)	CA/AA (mass ratio)
ZM-0	27.0	/	/	/
ZM-1	27.0	5.2	5.5	0.94
ZM-2	25.0	5.2	5.5	0.94
ZM-3	23.0	5.2	5.5	0.94
ZM-4	21.0	5.2	5.5	0.94
ZM-5	18.0	5.2	5.5	0.94
ZM-6	15.0	5.2	5.5	0.94
ZM-7	15.0	3.5	5.5	0.63
ZM-8	15.0	1.7	5.5	0.31
ZM-9	15.0	0.9	5.5	0.16
ZM-10	15.0	5.2	/	/
ZM-11	15.0	/	5.5	/
ZM-12	15.0	/	/	/
ZM-13	10.0	5.2	5.5	0.94