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Controlled release of protein from core–shell nanofibers prepared by emulsion electrospinning based on green chemical

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ABSTRACT: Direct application of active protein in practical food system suffers from the major disadvantage of protein inactivation caused by food components and environmental factors. This study is the first to encapsulate water-soluble protein into hydrophobic polystyrene (PS) polymer via emulsion electrospinning technique based on green chemical L-limonene to achieve sustained release of protein. Core–shell nanostructure with elongated domains was observed in electrospun fibers. *In vitro* release profiles suggest that a sustained release of protein was achieved. The increased release rate with PS molecular weight reveals that the release of protein could be well tuned by tailoring polymeric molecular weight, which is possibly attributed to the association of release rate with fiber inner structure and protein distribution in matrix. These results demonstrate that an excellent protein delivery system could be obtained via emulsion electrospinning based on green chemical for the application in antimicrobial packaging and filtration in food industry.
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INTRODUCTION

In the past decades, increasing attention has been focused on exploring strategies for delivering proteins/drugs continually in a prolonged period and in a controlled pattern.[1,2] Controlled delivery systems are able to efficiently encapsulate bioactive agents and achieve their release at a desired rate satisfying the need of the practical system, in order to enhance their functional efficacy and economic effectiveness and avoid agent inactivation induced by surrounding conditions.[3] It has been found that controlled delivery systems, introducing sustained release, could improve the efficiency of antimicrobial agents and prolong the shelf life of food systems during food processing and storage.[4] The primary approach to establish controlled delivery system is encapsulating bioactive agents into polymers, and the controlled release could be accomplished via modulating environment temperature,[5] formula of copolymer,[3] concentration of agent,[6] molecular weight of polymer and size of microspheres,[7] and so on. Until now, various biodegradable or nonbiodegradable polymers have been used to produce tunable carriers of bioactive ingredients.[8–10]

Electrospinning is widely recognized as an inexpensive, effective, simple, and versatile technique to fabricate ultrafine fiber mats to encapsulate bioactive molecules.[11,12] Due to the submicron diameter scale and high surface area to volume ratio, electrospun fibers containing bioactive agents could sufficiently contact with surrounding medium, making them an ideal material for tissue engineering, protein delivery systems and enzyme immobilization.[13–15] However, several major drawbacks in traditional blend electrospinning process limit its application in building controlled delivery systems for food-based applications. First, the simple blending of protein with polymer solution can induce their agglomeration either near or on the surface of fibers, which could give rise to severe initial burst release and result in reduction of the effective lifetime of devices and toxicological ramifications.[16,17] Second, long-time direct exposure of protein to adverse chemical environment of organic solvents in the preparation process could cause potential inactivation and denaturation of bioactive substances.[18] Besides, the traditional electrospinning method still faces enormous challenges to encapsulate water-soluble bioactive molecules into hydrophobic polymers. In recent years, coaxial electrospinning has been explored to prepare fibers to encapsulate dual agents by electrospinning two different solutions synchronously. However, the technique needs special apparatus, careful selection of operational parameters, complicated procedure and extra costs.[19,20]

Recently, emulsion electrospinning has gained growing interest in fabricating core–shell structured nanofibers for controlled

Additional Supporting Information may be found in the online version of this article.

release of water-soluble bioactive substances.[21–24] Specifically, water-in-oil (W/O) emulsions containing bioactive agents in water phase can be electrospun into core-sheath structured fibers with bioactive molecules encapsulated in core section, rather than uniformly dispersed inside the fiber.[24] By this means, a number of attractive advantages could be accomplished. Firstly, it can provide an effective approach for incorporation of water-soluble bioactive agents into hydrophobic polymers/organic solvents, overcoming the poor solubility and affinity of hydrophilic agents in water-insoluble polymers. Secondly, encapsulating bioactive agent in the core of the structure could avoid the negative effect of organic solvent, one of the major contributors to denaturation of bioactive agent. Thirdly, during the release process, the encapsulated agent needs to pass through the core/shell fiber matrix before entering medium, whereby the release rate could be alleviated to prolong the time period of release.[25] To date, Li *et al.* successfully encapsulated water-soluble proteinase K in poly(ethylene glycol)-poly(L-lactide) fiber by emulsion electrospinning and sustained release of the protein from fiber matrix was observed.[26] Besides, Yang *et al.* incorporated lysozyme into core-sheath structured poly(DL-lactide) fibers via emulsion electrospinning, where the release of protein lasted for up to 2 weeks.[27] However, the influence of operation parameters, for example, molecular weight of polymers, on the structure of core–shell structured emulsion electrospun fibers, as well as release behavior of protein from the fibers is still unclear.

Polystyrene (PS), a hydrophobic, air-stable, stiff, inexpensive, and biocompatible polymer, is one of the most widely used plastics in food industry, with annual consumption up to several billion kilograms.[28] Based on its resilience and inertness, it could be used for food packaging, filtration, insulation, etc.[29,30] PS could also be electrospun into ultrafine fibers by electrospinning technique via a wide variety of organic solvents, including tetrahydrofuran (THF), dimethylformamide (DMF), and dimethylacetamide (DMAc).[30,31] However, the organic solvents used in the electrospinning process can cause environmental problems and difficulties of process handling. Besides, the toxicity of these solvents limits the application of PS electrospun fiber in food area. Hence, in order to avoid the use of toxic solvents in the production of food-related products, PS used in electrospinning for food-based application should be dissolved in a natural and nontoxic solvent. In this regards, L-limonene, a safe, highly effective, and biodegradable natural extraction from peel of citrus fruits, is a good solvent alternative for the production of PS fiber matrix by electrospinning.[32] In 2005, Shin *et al.* successfully obtained PS electrospun nanofibers via dissolving waste PS in limonene.[32] However, literature lacks any inclusive report describing the encapsulation of hydrophilic proteins into PS fibers by emulsion electrospinning based on green solvents.

In this study, we described a novel approach to fabricate controlled delivery systems via emulsion electrospinning for sustained release of a water-soluble protein for food-related application. Four polystyrenes with a series of molecular weights were selected to investigate the influence of molecular weight on the morphology of electrospun fibers and release profiles of protein. L-limonene was applied as an organic solvent of PS during the electrospinning to avoid residue of toxic solvent and reduce environmental problems. Bovine serum albumin (BSA) was chosen as a model protein to evaluate the release profile of the desired delivery system.

EXPERIMENTAL

Materials

Polystyrenes with $M_w = 192{,}000$ g mol^{-1} (PS_{192k}), 280,000 g mol^{-1} (PS_{280k}), and 350,000 g mol^{-1} (PS_{350k}) were purchased from Sigma-Aldrich, respectively. Polystyrene with $M_w = 75{,}000$ g mol^{-1} (PS_{75k}) was purchased from Polymer Source, SPAN^{TM} 80 DMI-PC (Span 80) was obtained from Croda. One-side-polished silicon wafers of 4 in. diameter with orientation of <100> and resistivity of 0.001–0.005 Ω cm were purchased from the Institute of Electronic Materials Technology. All other chemicals were purchased from Aldrich and used without further purification. Ultrapure Milli-Q water (Millipore) with a resistivity of ~18 MΩ cm^{-1} was used in all experiments.

Preparation of Electrospinning Emulsions

Oil solution was prepared by dissolving 0.24 g Span 80 and 6 g PS_{75k} into 12.96 g L-limonene with 2 h of stirring. BSA was dissolved in Milli-Q water to obtain 40 mg mL^{-1} aqueous solution. 0.8 g aqueous phase was added into the oil solution and then emulsified at 13,500 rpm by an IKA T-25 ULTRA-TURRAX High-Speed Homogenizer (IKA Works) for 2 min to obtain uniform W/O emulsion. The same process was repeated for PS_{192k}, PS_{280k} and PS_{350k}. The droplet size and size distribution of W/O emulsion were measured by dynamic light scattering using Zetasizer Nano-ZS equipment (Malvern Instruments).

Emulsion Electrospinning

Two milliliters of emulsion was loaded into a 3 mL syringe with a 22 G blunt-end metal needle and mounted on to a syringe pump (New Era Pump Systems). The needle was connected with a high-voltage statitron and the supplied voltage was set at 20 kV. A grounded aluminum foil collector was placed 10 cm away from needle tip and the feeding rate was controlled at 0.2 mL h^{-1}. After being conducted at room temperature with atmosphere conditions for 4 h, the fiber mats were dried with a vacuum drying apparatus for 5 h to remove L-limonene residue. The schematic diagram of electrospinning process was showed in Figure 1.

Characterization of Electrospun Fiber Mats

Scanning Electron Microscopy. Scanning electron microscopy (SEM) images were obtained by using a Zeiss Auriga dual-beam FIB-SEM. Specifically, silicon wafers were cleaned by exposure to UV radiation for 2 h, immersed in concentrated H_2SO_4 for 15 min, rinsed with Milli-Q water, immersed in 0.1 M NaOH solution for 5 min, and finally thoroughly rinsed with Milli-Q water prior to electrospinning. Fibers were directly electrospun on silicon wafers and then sputter-coated with gold for analysis. Average fiber diameters were determined by measuring ~50 random fibers from the SEM images.

Atomic Force Microscopy. Surface images of electrospun fibers were observed with atomic force microscopy (AFM). All fibers were directly electrospun on silicon wafers. A NSCRIPTOR^{TM}

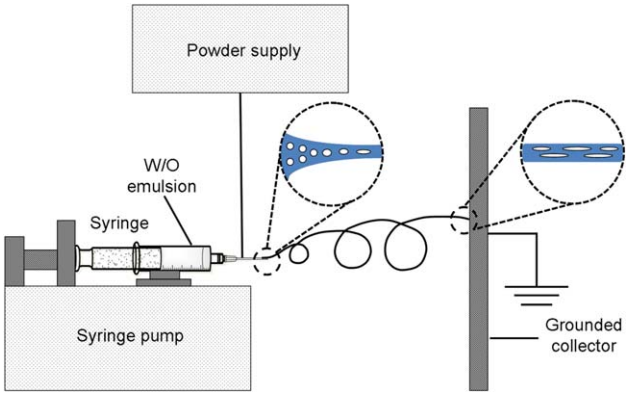

Figure 1. Schematic representation of emulsion electrospinning process. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

dip pen nanolithography system (Nanoink) operating in the AFM mode was used to characterize electrospun fibers. AFM was operated in contact mode by P-MAN-SICT-0 AFM cantilevers (Pacific Nanotechnology) with a nominal force constant of 0.2 N m^{-1}.

Transmission Electron Microscopy. Transmission electron microscopy (TEM) images were measured using a FEI CM20 field emission microscope, operated at a voltage of 200 kV. To prepare TEM samples, fibers were directly spun on carbon-coated copper grids and kept in a vacuum oven at room temperature for 48 h.

Protein Loading in the Electrospun Fiber Mats. The protein content in the nanofiber was determined by ^{1}H-NMR in $CDCl_3$ (shown in Figure S1 in Supporting Information). Briefly, BSA/PS weight ratio in the matrix was obtained by the integral area ratio of the peaks at 4.7 (amide protons of BSA) and 7.0 ppm (aromatic protons of polystyrene) in ^{1}H-NMR spectrum of the fiber mats. The BSA loading percentage was calculated according to BSA/PS/Span 80 feed ratio of standard sample. The BSA loading in the finished fibers were 0.5 wt % and there was no noticeable difference in BSA loading between the PS fibers of four molecular weights.

Contact Angle Measurements. In order to investigate the influence of PS molecular weight, emulsion droplet, and BSA incorporation on the hydrophobicity of fiber mats, water contact angle of electrospun PS fibers fabricated with BSA emulsion and empty emulsion (in absence of BSA), as well as blank PS fibers were measured. A water drop was placed onto the surface of fibers and contact angle was observed with a contact angle apparatus (VCA-optima, AST) at room temperature. Contact angles were calculated automatically by VCA-optima software. Values are an average of 15 measurements taken on different areas of the fiber mats.

Determination of Protein Release Profiles from Fiber Mats. BSA loaded PS fiber mats (100 mg) were placed in 2 mL phosphate buffered saline (PBS) (pH = 7.0) and incubated in an orbital shaker at room temperature. 0.5 mL of PBS was taken from each tube for analysis at specific time intervals and an equal volume of fresh PBS was back-added to maintain the total volume constant. The concentration of BSA was measured with BCA kit. The experiments were carried out in triplicate. The cumulative release of BSA was calculated with the following equation:

$$R_{t_i}(\%) = \frac{\left(\sum_{n=1}^{i-1} a_n + 4a_i\right)}{U_0} \times 100\%$$

where R_{t_i} (%) represents the cumulatively released BSA at time t_i, the ith time of sampling; a_i is the concentration of BSA in the released buffer at the sampling time t_i; and U_0 is the total encapsulated BSA in 100 mg fiber (corresponding to 100% release).

RESULTS AND DISCUSSION

The retention of encapsulated protein activity is mainly attributed to the dissolution of protein in water phase, and its sustained release achieved by the core–shell structure, by which the inactivation resulted from the practical environment could be avoided.[25] Maretschek *et al.* incorporated protein cytochrome C into poly(L-lactide) by means of emulsion electrospinning to control protein release via varying the proportion of polymer in formula.[33] In 2005, Jiang *et al.* encapsulated lysozyme into polycaprolactone (PCL) nanofibers, where polymer was dissolved in the mixture of DMF and chloroform, indicating that a sustained release of protein could be successfully achieved.[19] Although a number of researchers have investigated controlled delivery systems via emulsion electrospinning, little information is available on the encapsulation of water-soluble proteins in PS polymer via nontoxic solvent. For the first time, we encapsulated water-soluble protein in the core–shell structure via emulsion electrospinning based on nonbiodegradable but biocompatible PS polymer and a natural organic solvent—L-limonene.

Physicochemical Properties of Electrospun PS Fiber Mats

The formation of electrospun fibers during electrospinning is a consequence of the balance among electric field, surface tension, and viscoelastic forces. Polymeric molecular weight has been known to have significant effect on the structure of the electrospun fiber matrix.[34–36] SEM micrographs of BSA loaded electrospun PS fiber mats were revealed in Figure 2. Ultrafine fibers with nano-scale diameters could be observed in SEM images, indicating that L-limonene could be used to dissolve PS polymer for electrospun fiber fabrication. Produced by PS polymer with the lowest molecular weight (75 kDa), fiber presented bead-and-string structured morphology [Figure 2(A)]. Likewise, Qi *et al.* observed the similar bead-and-string structure when encapsulating protein into poly(L-lactic acid) via emulsion electrsopinning.[21] According to the study of Kim *et al.* this phenomenon might be caused by the insufficient stretch of the polymer jet during electrospinning.[37] With the same concentration (30%), PS_{75k} polymer possesses lower viscosity than the other ones, resulting in the insufficient stretching and bead-and-string structure during fiber solidification. As the molecular weight increased, fully fibrous structure could be produced, as shown in Figure 2(B–D). The average diameters of PS_{75k}, PS_{192k}, PS_{280k}, and PS_{350k} fibers were 596 ± 58, 868 ± 96, 1175 ± 106, and 1385 ± 162 nm, respectively. The results were in good agreement with those obtained from AFM images in

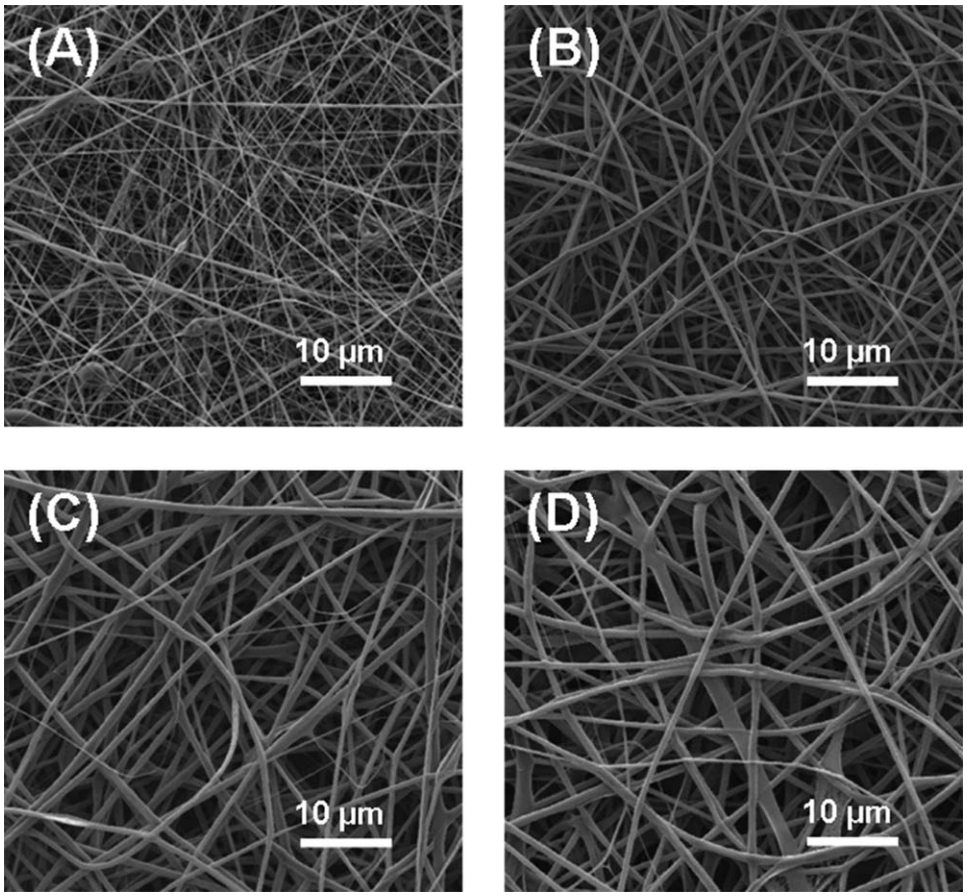

Figure 2. SEM images of BSA loaded electrospun nanofibers with various molecular weights of PS. The molecular weight of PS: (A) 75 kDa; (B) 192 kDa; (C) 280 kDa; (D) 350 kDa, respectively.

Figure 3. The observed increase in the diameters of electrospun fibers with PS molecular weight (Figure 4) was likely attributed to the enhanced viscosity of emulsions. It is known that PS polymer with higher molecular weight possesses increased viscosity value, which could lead to larger size of electrospun fiber.[38,39] Additionally, the droplet size of emulsion also offers significant influence on the fiber sizes.[22] The fiber with larger diameter can be obtained from the emulsion with larger droplet size. According to Figure 5, larger emulsion droplet size could be observed in W/O emulsion with PS of higher molecular weight, which induced the enlargement of fiber diameters. The results agreed well with those in the previous findings.[22,24]

TEM observation was conducted to investigate the inner structure of BSA loaded electrospun nanofibers (Figure 6). The elongated small and large domains with sharp boundaries between core and shell components were observed in the matrix of BSA loaded electrospun PS nanofibers, indicative of the difference of electron absorption ability between the core and shell materials. The core and shell sections were possibly composed of BSA in aqueous phase and PS in oil phase of emulsions, respectively.[26] Similar entrapment of water pockets in polymer fibers during emulsion electrospinning process has been previously reported.[22,40] The likely reasons for the formation of elongated domains in the matrix of the composite fibers may be associated with the deformation of water droplets at the Taylor cone along with the surrounding PS/L-limonene solution and their breakup into smaller droplets during polymer fiber formation via a Rayleigh instability. This phenomenon is frequently observed in liquid-within-liquid columns and elongated droplets of polymers in electric fields.[40] During the emulsion electrospinning process, the jet of liquid fluid underwent stretching and bending when the emulsion emerged from the tip of a small-bore nozzle. The suspended water droplets moved perpendicularly from the surface into the center and accumulated in the axial region due to the rapid evaporation of L-limonene organic phase and elongation of the polymer matrix. As L-limonene evaporated faster than water, the viscosity of polymer organic solution in outer layer was increased more rapidly than that of BSA aqueous phase in inner layer. The contrast in viscosity of the two phases and their immiscibility drove the aqueous phase to interpenetrate into the fiber interior rather than on the surfaces. The emulsion droplets further broke up and stretched into multiple cores with an elliptical shape such that the core-sheath structure can be observed in fibers from TEM images.[23] The viscosity of the primary organic phase of W/O emulsion could strongly influence the inner structure of electrospun fibers.[41] With the increase in the polymeric molecular weight, the viscosity of the primary organic phase of emulsion was enhanced and the movement of molecules could be decelerated. Therefore, use of higher molecular weight polymers led to slower inward

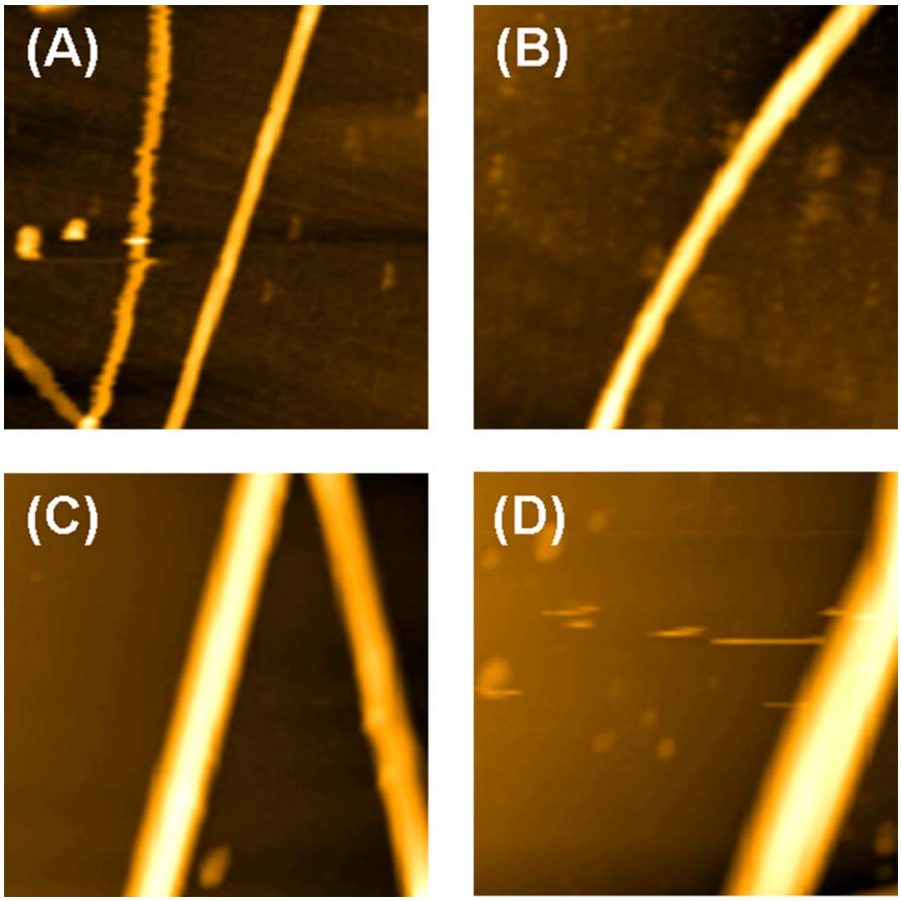

Figure 3. AFM topography images for BSA loaded electrospun nanofibers with various molecular weights of PS. The molecular weight of PS: (A) 75 kDa; (B) 192 kDa; (C) 280 kDa; (D) 350 kDa, respectively. Scale: 10 × 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

movement of emulsion droplets during electrospinning process so that the water droplets with protein located close to, or loosely associated with, or even absorbed on the fiber surface. The irregular and individual elongated droplets in fibers formed in the direction of fiber trajectory as illustrated by TEM. The differences in inner structure and diameters of fibers would likely exhibit significant influence on the surface energy of fiber mats and release profiles of encapsulated BSA proteins during the incubation.

Wettability of nanofibrous mats plays a critical role in their physical property and applications in tissue engineering, food

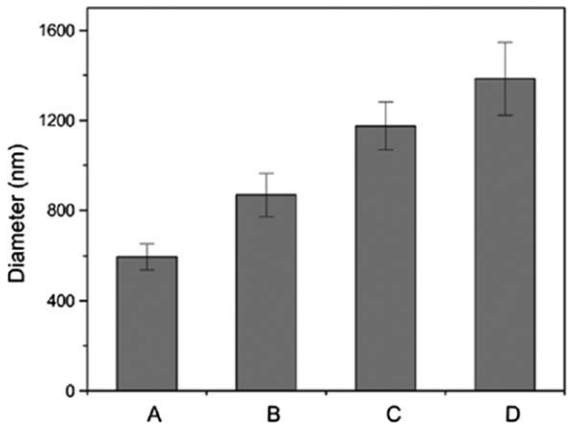

Figure 4. Average diameters of BSA loaded electrospun nanofibers with various molecular weights of PS. The molecular weight of PS: (A) 75 kDa; (B) 192 kDa; (C) 280 kDa; (D) 350 kDa, respectively.

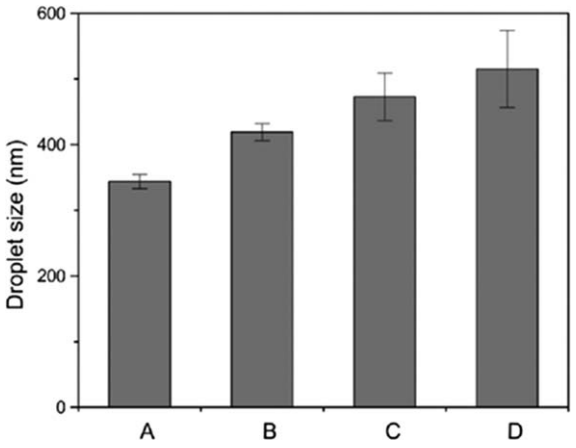

Figure 5. Average droplet sizes of BSA/Span80/PS W/O emulsion with various molecular weights of PS. The molecular weight of PS: (A) 75 kDa; (B) 192 kDa; (C) 280 kDa; (D) 350 kDa, respectively.

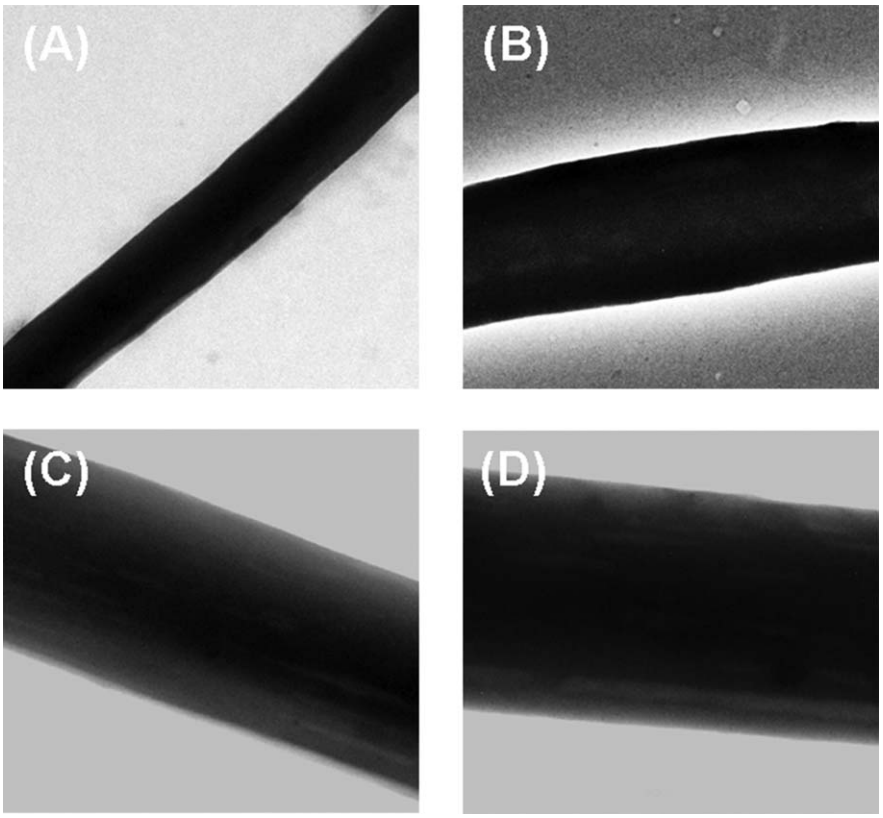

Figure 6. Transmission electron microscopy images of core-sheath structured PS nanofibers encapsulated with BSA. The molecular weight of PS: (A) 75 kDa; (B) 192 kDa; (C) 280 kDa; (D) 350 kDa, respectively. Scale: 2 × 2 μm.

packaging and other fields.[42] According to the study of Kang *et al.*, surface energy of fiber mats is mainly determined by two pivotal factors—the roughness of surface morphology and hydrophobicity of chemical composition. Specifically, a rough surface morphology and chemically hydrophobic components of PS fiber mats could lead to enhanced hydrophobicity of the solid surface.[43] Additionally, as early as in 1944, Cassie and Baxter revealed that air trapped at a protuberant surface could hold up the water droplet during the contact angle measurement, showing great impact on the hydrophobicity of fibers.[44,45]

As shown in Figure 7, water contact angles of four pure fibers of PS_{75k}, PS_{192k}, PS_{280k}, and PS_{350k} were 126.6° ± 4.8°, 126.5° ± 4.8°, 124.5° ± 3.7°, and 128.4° ± 2.9°, respectively, while those of PS fibers with BSA were 133.5° ± 3.2°, 133.5° ± 3.1°, 133.1° ± 2.8°, and 132.8° ± 2.32°, respectively. It is noted that four PS fibers possessed nearly identical water contact angles, indicating that molecular weight of PS polymer have no effect on water contact angle of fibers. Kang *et al.* have acknowledged that contact angle was greatly related to the organic solvent used to dissolve PS.[43] Enhancement in contact angle presented in the fibers incorporated with BSA as compared with that of blank PS fibers. In order to investigate the possible reason for the change of fiber hydrophobicity, the wettabilities of blank PS_{192k} fiber, PS_{192k} fiber with empty emulsion (in absence of BSA) and PS_{192k} fiber with emulsion containing BSA were measured. According to Figure 8, fiber fabricated with empty emulsion exhibited almost the same contact angle as fiber containing BSA, where both were higher than that of blank PS fiber. Combining Figures 7 and 8, it can be implied that water droplets rather than BSA in emulsion caused the enhancement in hydrophobicity of PS fibers. Although small part of hydrophilic BSA was possibly randomly distributed on the surface of fibers, which might lead to the reduction of contact angle, the effect of this factor was overcame by the influence of water droplets in W/O emulsion.[46] During emulsion

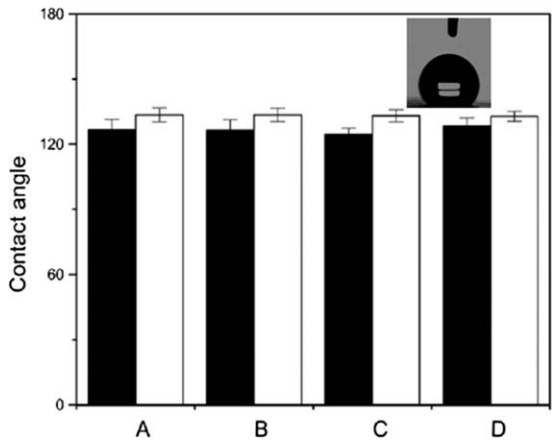

Figure 7. Water contact angle of blank PS fiber (solid column) and BSA loaded PS fibers (blank column). The molecular weight of PS: (A) 75 kDa; (B) 192 kDa; (C) 280 kDa; (D) 350 kDa, respectively.

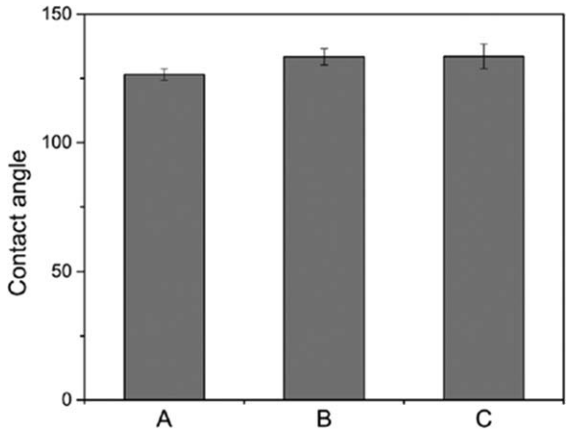

Figure 8. Water contact angle of electrospun fiber mats fabricated by (A) PS/L-limonene solution, (B) W/O emulsion without BSA in PS/L-limonene mixture, and (C) W/O emulsion with BSA in PS/L-limonene mixture, respectively. The molecular weight of PS is 192 kDa.

electrospinning process, air could be trapped near the surface of fiber after the evaporation of water droplets and intrigued some predictions at surface structure, resulting in the enhancement of contact angle.[43,47] On the other hand, numerous protuberances were possibly introduced by phase separation of aqueous and organic phases in the fibers, and rough morphology formed at the surface of fibers, as aforementioned, dominated in their high hydrophobicity.

In Vitro Release of BSA from Electrospun Fibers

Core–shell structured fibers formed via emulsion electrospinning, wherein proteins were encapsulated in the core of the fiber mats, could serve as the reservoirs for the proteins and provide sustained release profile.[48] The cumulative release of BSA from four types of electrospun nanofiber mats in PBS at room temperature was assessed by BCA kit, and the release profiles were shown in Figure 9. The entire release process could be split into two stages—initial burst release and subsequent stable release. With the same encapsulation efficiency of BSA in PS electrospun fibers (Supporting Information Figure S1), modest burst release was presented in all samples within the first 2 days, accounting for 17, 28, 34, and 41% for fibers of PS_{75k}, PS_{192k}, PS_{280k}, and PS_{350k}, respectively. Afterwards, the protein release were in a relatively steady manner and sustained for more than 50 days, reaching 58, 76, 93, and 96% at the end of incubation period, respectively. No changes in structure of fibers have been observed after BSA release from TEM images (shown in Supporting Information Figure S2). It is notable that the release of protein from PS_{75k} and PS_{192k} fibers still went on at the end of test. In addition, both of release behaviors of PS_{280k} and PS_{350k} fibers were almost identical, with nearly complete release at ~50 days. These results revealed that BSA could be efficiently encapsulated in the fibers and sustainably released from the matrix. Additionally, the release profiles of protein from emulsion electrospun PS fibers varied obviously corresponding to PS molecular weight, indicating the feasibilities of achieving controlled release by tailoring molecular weight of polymer.

The initial burst release could be explained by two facts of matters. Under the electrical driving force during electrospinning process, a certain portion of positively charged BSA agglomerated close to or upon the surface of fibers. Once immersed in PBS, most of BSA presented on or adjacent to the surface was released immediately.[25,46] On the other hand, as mentioned in the previous studies, the burst release of encapsulated proteins in fibers might be attributed to the diffusion of BSA out of the core structure through the imperfections of core–shell structure, such as the shell failure and the porous structure to favor good permeability.[27] Thereafter, protein encapsulated in the core was released through the scaffold or pores therein via diffusion mechanism and thereby generating sustained release. This explanation was supported by the study of Jiang *et al.* who investigated the release behavior of protein from poly(ε-caprolactone) electrospun fiber with core–shell structure and disclosed that the release mechanism of incorporated proteins was dominantly based on the molecular diffusion in the matrix.[19] Likewise, Yang *et al.* also suggested the similar release mechanism of lysozyme from poly(DL-lactic acid) nanofiber.[27]

Polymer molecular weight is one of the key factors affecting the release of encapsulated ingredients.[49] As shown in Figure 9, sustainability of BSA release from nanofibers was varied depending on the molecular weight of polymeric matrix. Interestingly, the fiber with the lowest molecular weight PS (75 kDa) showed enhanced sustained release, while fibers produced by high molecular weight (280 and 350 kDa) PS displayed faster protein release rate, respectively. This result agreed well with the findings that lower molecular weight of polymer resulted in slower release rate of protein from microspherical delivery systems.[7] The dependence of release rate on molecular weight of PS might be a direct consequence of the combination of the distribution of BSA protein and inner structure of fibers, as well as the porosity of fiber shell matrix.

The distribution of protein in fiber matrix plays an important role in the release profiles. During the fiber solidification, the dispersion of protein in the fiber during electrospinning process mainly depends on the solvent evaporation rate. It is possible that the increase in viscosity simultaneously with increased molecular

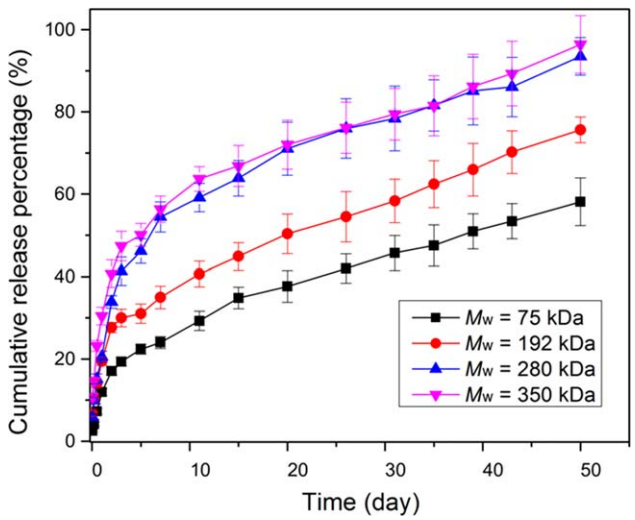

Figure 9. Release profiles of BSA from electrospun nanofibers with PS of various molecular weights in PBS (pH = 7.0) at room temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

weight could reduce the evaporation rate of solvent. The low evaporation rate of solvent could lead to slow polymer solidification and inward movement of water droplets. As a result, BSA accumulated close to the surface of fiber wall so that it was prone to rapid diffusion out of fiber matrix during release process and thus demonstrated enhanced release rate.[38,50]

Along with protein distribution, inner structure is another major contributor for tailoring release behavior of protein, because the diffusion of protein largely depends on the density and porosity of fibers. With the increase of molecular weight, the diffusion coefficient of solute was reduced, which could weaken the intermolecular entanglements and cause the increase of porosity degree of fiber wall. Besides, increase in molecular weight reflected longer polymer chain, leading to enhanced steric hindrance of polymer backbone. Both of the factors could contribute to a higher porosity degree in polymer fiber matrix. As a consequence, BSA diffused more easily through the looser matrix with polymer of higher molecular weight, generating a fast release of protein from fibers.

CONCLUSIONS

In conclusion, emulsion electrospinning was successfully introduced to produce core–shell-structured ultrafine PS fibers, where green chemical L-limonene was utilized as solvent to eliminate toxicity introduced by organic ones. By means of emulsion electrospinning, the initial burst release of water-soluble protein was pronouncedly alleviated and the release period could be prolonged to over 50 days and well controlled by tailoring the molecular weight of PS polymers. PS with higher molecular weight provided faster release of protein due to the agglomeration of BSA on or adjacent to the surface of fiber and the looser fiber matrix. Current results indicated the feasibility of encapsulation of water-soluble protein in PS nanofibers with natural solvent L-limonene by means of emulsion electrospinning technique. It provides a basis for further exploration to achieve highly sustainable, controllable, and effective protein-releasing kinetics of bioactive proteins with green chemical, which would find better applications of electrospun fibers in food fields. As active protein can be inactivated by food components and environmental factors when directly applied in practical food system, further research focus should be on the application of this developed release system in antimicrobial protein encapsulation to improve their activity in food systems.

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