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Controlled release of a hydrophilic drug from electrospun amyloid-like protein blend nanofibers

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ABSTRACT

In this study, a controlled drug release platform, amyloid-like bovine serum albumin (AL-BSA) with ampicillin sodium salt (amp), was developed. To develop this platform, 5%, 10%, and 20% (w/w) ratios of amp:BSA were used with electrospinning to prepare nanofibers with average diameters of 132 ± 69, 159 ± 60, and 179 ± 42 nm, respectively. Fourier transform infrared spectroscopy demonstrated that AL-BSA could entrap large amounts of drug inside the nanofibers, which was attributed to the antimicrobial activity of the released drug against *Escherichia coli* and *Staphylococcus aureus*. The amount of drug released was measured by UV-VIS spectrophotometry. The nanofibrous matrix of the electrospun membrane showed controlled release behavior in all samples. The transport mechanism was Fickian for the low ratio of amp:BSA (5% w:w). When the drug ratio was increased to > 10% (w:w), thicker fiber structures formed, suggesting that the drug traveled a longer distance to reach the fiber surface; thus, the mechanism of transport shifted from Fickian to non-Fickian.

1. Introduction

Natural polymer-based self-assembled nanomaterials have gained attention for various biomedical applications, particularly because they share many properties with molecules in the human body and show biocompatibility and biodegradability. For controlled drug delivery, the nanostructure morphology of natural membranes may have several advantages compared to bulk films.

Electrospinning is a method for producing nanofibers with diameters varying from the micro to the nanoscale. In contrast to conventional fiber production techniques, it relies on self-assembly processes driven by Coulomb interactions between charged elements of the polymer solutions to be spun into nanofibers [1]. Synthetic or natural polymers can be used to produce nanofibrous materials for various fields such as filtration, biosensors, tissue regeneration, wound dressings, and drug delivery [2–7].

Electrospun nanofibers show high potential for drug delivery because of their large surface area, high porosity, and porous structure. Additionally, the tractable morphology and composition of the nanofibers allows for controlled drug release. Several controlled drug release profiles such as sustained, burst, and delayed can be obtained by using electrospun nanofibrous membranes as carriers for model drugs [8–11]. The ability to adhere the scaffold at the site of infection is advantageous compared to other drug delivery vehicles [12,13].

Electrospinning of natural polymers is more advantageous than that of synthetic polymers for biomedical applications because of their similarity to molecules in the human body [14]. To take the advantage of this biological similarity, natural polymers such as peptides [15], chitin [16], cellulose [17], and collagen [18–20] have been used for drug delivery with electrospinning such as by combining these materials with another polymer by blending or producing core-shell structures [21].

To produce drug-loaded nanofibrous composite membranes with a single needle, the model drug and polymer should be dissolved together and the resulting mixture should be electrospun *via* single electrospinning. However, the initial burst release is indispensable for such membranes because of the drug distribution on the surface of the nanofibers, large nanofiber surface areas, and amorphous status of the drugs inside the nanofibers [22].

In this study, amyloid-like protein membranes containing with a hydrophilic drug were produced by single electrospinning for drug delivery. To achieve this, the natural polymer bovine serum albumin (BSA) was used as a supporting drug carrier, while ampicillin (amp) was used as a hydrophilic model drug. The effect of drug concentration

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in nanofibrous membrane was studied to investigate the release behavior of ampicillin. The results of this study demonstrated that, the application area of single electrospinning could be expanded to natural polymers for the potential controlled release of hydrophilic drugs.

2. Materials and methods

2.1. Materials

Ampicillin sodium salt (amp), 2,2,2 trifluoroethanol (TFE), β-mercaptoethanol (β-ME), and phosphate-buffered saline (PBS) tablets (pH 7.4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). BSA was supplied by Acros Organics (Geel, Belgium). Double-distilled water was used in all experiments.

Staphylococcus aureus was purchased from American Type Culture Collection (Manassas, VA, USA) in the form of a freeze-dried culture (Cat. No. 33592, Lot No. 2436360) and were reconstituted in nutrient broth (Sigma, Cat. No. N7519). *Escherichia coli* BL21 was obtained from Novagen (Madison, WI, USA). Brain Heart Broth medium was supplied by Merck (Kenilworth, NJ, USA).

2.2. Electrospinning

BSA was dissolved in a mixture of TFE and PBS to obtain a 12% (w/v) final solution of amyloid-like BSA (AL-BA) at room temperature (25 °C). As reported previously, the tertiary structure of the protein was decomposed using β-ME and stabilized by the addition of TFE [3,23]. BSA containing solvent was stirred continuously at for 4 h. After obtaining a homogenous solution of BSA, ampicillin was added at weight ratios of 5%, 10%, and 20% and then stirred to procure the final electrospinnable solution. Viscosity measurements of solvents and solutions were conducted at 50 rpm and 28 °C with a viscometer (Brookfield, LV-DVIP, Middleboro, MA, USA) in a small sample adapter (Brookfield, Middleboro, MA, USA).

A vertically fixed 5-mL syringe with a metal needle, with an inner diameter of 0.80 mm, was filled with amp-BSA solution. In the vertical setup, the aluminum collector was placed vertically and the syringe pump was located above the collector. For the electrospinning setup, a direct current (DC) voltage supplier (MCH 303D2; Gamma High Voltage Research Inc., Ormond Beach, FL, USA) and syringe pump (NE-1000, New Era Pump Systems, Farmingdale, NY, USA) were used. The parameters generating a stable Taylor cone and uniform nanofiber distribution were determined after optimization of the solution and working parameters such as drug concentration, processing voltage, and flow rate. In our preliminary studies, the flow rates were maintained between 0.20 and 0.60 mL/h, applied voltage varied from 12 to 24 kV under a constant tip-to-collector distance of 11 cm, respectively, and fibers were collected for 4 h. All experiments were performed at room temperature and the samples were stored in a vacuum desiccator overnight prior to use. A schematic diagram for the experimental setup is shown in Fig. 1.

2.3. Characterization of ampicillin-loaded amyloid-like BSA nanofibers

For preliminary studies, electrospun fibers were collected on microscope slides and observed under a high-resolution polarized light microscope (Nikon Eclipse, LV100, Tokyo, Japan). An environmental scanning electron microscope (e-SEM; FEI-Quanta 200 FEI, Hillsboro, OR, USA) was used to investigate the morphology of the membranes containing 5%, 10%, and 20% (w:w) amp:BSA. Prior to examination, the samples were sputter-coated with Au. ImageJ® software (NIH, Bethesda, MD, USA) was used to measure the average fiber diameter of the fibrous networks from e-SEM images.

The compatibility between the drug and fibrous network for all membranes was investigated by attenuated total reflectance Fourier transform infrared (ATR-FTIR) analysis, which was performed using an FTIR spectrometer (Perkin Elmer Spectrum, 100, Waltham, MA, USA). Spectra were collected in the range of 500–4000 cm^{-1}.

The thermal stability properties of 5%, 10%, and 20% (w:w) amp:BSA membranes were analyzed using a TGA (thermal gravimetric analysis, Q500 V20.13 Build 39, TA Instruments Co., New Castle, DE, USA) at a heating rate of 10 °C/min under a nitrogen atmosphere. For thermal stability analysis, 2–5 mg of sample was used. Derivative thermal gravimetric results were also obtained to determine the maximum rate of weight loss. Argon was used as a purge gas (10 mL/min). Freeze-dried samples were placed in aluminum oxide pans and heated from 20 to 600 °C at a heating rate of 10 °C/min. The degradation temperature (T_d) and weight loss at 600 °C were calculated using ORIGIN software (version 9.0 PRO, OriginLab Corporation, Northampton, MA, USA).

Differential scanning calorimetry (DSC) was performed on a TA Instruments system for each membrane. Argon was used as a purge gas (40 mL/min). Samples between 5 and 6.5 mg were placed in aluminum pan and heated from 20 °C to 250 °C at a heating rate of 10 °C/min. The thermal stability of pure ampicillin (amp), BSA, and amp:BSA membrane samples were evaluated in terms of decomposition temperature.

Moreover, contact angles for all produced membranes were determined by the sessile drop method after placing drops of test liquids onto the membrane surface using a module (KSV Instruments Ltd., Helsinki, Finland). Contact angles were measured at least three times at different locations on the AL-BSA and 5%, 10%, and 20% (w:w) amp:BSA membrane surfaces. The results were reported as the mean ± SD.

2.4. In vitro release studies

Each sample was cut into 1.5-cm^2 pieces, which were approximately 100 mg. These samples were immersed in 5 mL of 0.1 M phosphate-buffered saline (PBS) (pH 7.4), and stirred in a shaking incubator (Suzhou Weiner, China) at 100 rpm for 1–144 h. At predetermined time points (1, 2, 3, 4, 6, 8, 12, 24, 48, 96, 120, 144 h), 1 mL of this solution was removed and filtered; 1.0 mL fresh buffer solution was added to maintain the same total solution volume. Ampicillin concentration was measured using a UV-2012 spectrophotometer (Hitachi U-5100, Tokyo, Japan) at 204 nm. The cumulative release percentage of ampicillin was determined from a standard calibration curve. All release studies were carried out in triplicate for 10 different membranes in each group and the results are reported as the mean ± SD.

2.5. Antimicrobial activity

The antimicrobial properties of the NC1, NC2, and 5%, 10%, and 20% (w:w) amp:BSA membranes were tested against two model microorganisms: *Staphylococcus aureus*, which is gram-positive, and *Escherichia coli*, which is gram-negative. Sterile conditions were used during the experiment. For the antibacterial assays, cultures of *E. coli* and *S. aureus* were grown overnight in Brain Heart Broth medium in a 37 °C incubator for 24 h. The cultures were then diluted to obtain 10^8 CFU/mL solutions. The nanofibrous membranes were then cut into 2.5-cm^2 pieces of approximately 100 mg and placed in the center of a Brain Heart Agar plate coated with 100 μL of each bacterium. The plates were incubated at 37 °C for 24 h. Drug-free AL-BSA membranes were used as a negative control. Images of each sample were analyzed to determine the zone formation to determine the antimicrobial effect of ampicillin from 5%, 10%, and 20% (w:w) amp:BSA-containing nanofibrous membranes.

2.6. Drug release study

Because nanofibers have a large surface to volume ratio and short diffusion length, the degradation properties and drug release profiles of electrospun membranes are very different from bulk films with the