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Mussel-inspired protein-mediated surface functionalization of electrospun nanofibers for pH-responsive drug delivery

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ABSTRACT

pH-responsive drug delivery systems could mediate drug releasing rate by changing the pH values at specific times as per the pathophysiological need of the disease. This paper demonstrates that a mussel-inspired protein polydopamine coating can tune the loading and releasing rate of charged molecules from electrospun poly(ε-caprolactone) (PCL) nanofibers in solutions with different pH values. In vitro release profiles show that the positive charged molecules release significantly faster in acidic than those in neutral and basic environments within the same incubation time. The results of fluorescein diacetate staining and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays show the viability of cancer cells after treatment with doxorubicin-released media at different pH values qualitatively and quantitatively, indicating that the media containing doxorubicin that were released in solutions at low pH values could kill a significantly higher number of cells than those released in solutions at high pH values. Together, the pH-responsive drug delivery systems based on polydopamine-coated PCL nanofibers could have potential application in the oral delivery of anticancer drugs for treating gastric cancer and in vaginal delivery of anti-viral drugs or anti-inflammatory drugs, which could raise their efficacy, deliver them to the specific target and minimize their toxic side effects.

1. Introduction

Controlled drug delivery systems (CDDS) have been widely investigated for treating many diseases [1,2]. Normally, CCDS are developed based on two criteria. One is temporal drug modulation matching the physiological needs, and the other is drug distribution on a specific target [3]. Nano-scale materials are coming into prominence as a new generation of CDDS, which can be devised to tune release kinetics, to regulate biodistribution and to minimize toxic side effects, thereby enhancing the therapeutic index of a given drug [4]. Among them, electrospun nanofibers have demonstrated great potential for applications in the field of topical and oral drug delivery, owing to their unique features, including versatility of drug incorporation, high loading efficiency, high surface-area-to-volume ratio, flexibility in surface functionalities and effective cost [5,6]. So far, a number of bioactive materials have been incorporated into electrospun fibers for controlled release, including small molecular drugs, herbs, peptides, proteins, DNA and vaccines [7,8]. Kenawy et al. first reported electrospun fiber mats made from poly(lactic acid) (PLA), poly(ethylene-*co*-vinyl acetate) (PEVA) or from a 50:50 blend of the two as vehicles for tetracycline hydrochloride delivery. The drug release rate was controlled mainly by diffusion, owing to the slow degradation of PLA and the non-degradable property of PEVA [9]. Subsequent studies often used biodegradable materials for controlling rates of drug release from electrospun nanofibers through diffusion (i.e. polymers with slow degradation rates), degradation (i.e. polymers with fast degradation rates) or a combination of diffusion and polymer degradation (i.e. polymers with moderate degradation rates). Notable examples include biodegradable electrospun poly(L-lactic acid) fibers for rifampin delivery, poly(ε-caprolactone) (PCL) fiber meshes with the hydrophobic polymer dopant poly(glycerol monostearate-*co*-ε-caprolactone) (PGC-C18) for camptothecin-11 or irinotecan hydrochloride and 7-ethyl-10-hydroxycamptothecin delivery, fiber mats made of tyrosine-derived polycarbonate terpolymers for peptide delivery, and PLGA fibers for paclitaxel delivery [10–13].

In order to finely mediate drug release at specific times as per the pathophysiological need of the disease, stimuli-responsive drug delivery is very critical. Stimuli-responsive drug delivery systems are in the vanguard of drug administration, as they can respond to small signs and changes in the surrounding environment, which translate into significant changes in their microstructure and in the physiological and chemical properties, as desired [14,15]. The signs or stimuli that trigger the structural changes in

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these smart polymers can be classified into three main categories: physical stimuli (i.e. temperature, ultrasound, light and mechanical stress), chemical stimuli (i.e. pH and ionic strength) and biological stimuli (i.e. enzymes and biomolecules) [3]. Owing to the remarkable changes in pH value in the human body, pH-responsive drug delivery systems have been designed for carrying and directing therapeutic agents to a specific body area, tissue or organ. Representative examples include the use of poly(*N*, *N*-dimethylaminoethyl methacrylate) with amino groups and poly(acrylic acid) (PAA) with carboxylic groups for controlling drug release in acidic and alkaline pH environments, respectively [16,17]. So far, however, few studies have examined pH-responsive drug delivery systems composed of electrospun nanofibers, which could be due to the difficulty of fabrication of such smart fibers using the electrospinning technique. Chunder et al. [18] fabricated methylene blue-loaded ultrathin fibers composed of PAA and poly(allylamine hydrochloride) and demonstrated the controlled release of methylene blue (low molecular weight cationic molecules) from fibers by pH in non-buffered solutions based on the replacement of protons in carboxylate groups in fibers. In other studies, Qi et al. and Cui et al. [19,20] developed electrospun nanofibers of acid-labile biodegradable polymers containing ortho ester groups or acetal groups for pH-controlled release of paracetamol. In a separate study, Huang et al. [21] demonstrated the incorporation of anti-viral drugs in electrospun cellulose acetate phthalate fibers: the fibers were stable in healthy vaginal fluid (pH < 4.5) and become dissolvable immediately upon addition of a small amount of human semen (pH between 7.4 and 8.4), which caused the release of encapsulated drugs, indicating the potential for prevention of human immunodeficiency virus transmission [21].

The present authors recently reported polydopamine-mediated surface modification to electrospun PCL and PLA nanofibers for biomedical applications, including tissue engineering and sustained drug delivery [5]. In addition, previous studies showed that weak chemical bonds present on PCL fibers can be replaced by highly reactive carbony (−CO−), carboxyl (−COOH), and hydroxyl (−OH) groups after oxygen-containing plasma treatment [22–24]. Further, recent studies demonstrated that polydopamine coating had selective permeabilities for charged molecules under different pH values [25]. The objective of the present study was to develop pH-responsive drug delivery systems based on polydopamine-coated electrospun nanofibers. It was hypothesized that the release of charged molecules from air-plasma-treated electrospun PCL nanofibers could be responsive to different pH values. It was further hypothesized that the additional polydopamine coating could finely tailor the pH-responsive release of charged molecules. This study showed that uptake and release rates of both rhodamine 6G hydrochloride (R6G) and doxorubicin hydrochloride (DOX) can be mediated by the solution's pH value. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay of H1299 was conducted using a DOX-releasing cell culture medium, indicating that DOX-releasing medium could kill more cancer cells at low pH values than at high pH values. This showed that polydopamine-coated PCL (PDPCL) nanofibers may have potential application in oral and topical drug delivery to targets where it is related to changes in pH values.

2. Materials and methods

2.1. Fabrication of electrospun fibers and polydopamine coating

Fibers were produced using a standard electrospinning setup as described earlier [5], PCL (M_w = 80 kDa; Sigma–Aldrich, St. Louis, MO) was dissolved in a solvent mixture consisting of dichloromethane (DCM; fisher chemical, Waltham, MA) and *N*,*N*-dimethylformamide (fisher chemical, Waltham, MA) in a ratio of 4:1 (v/v) at a concentration of 10% (w/v). PCL solutions were pumped at a flow rate of 0.5 ml h^{-1}, using a syringe pump, while a potential of 12 kV was applied between the spinneret (a 22-gage needle) and a grounded collector located 12 cm apart from the spinneret. Random fibers were collected by a stainless steel drum, which rotated at <100 rpm. The fiber mats were then treated with an air plasma by a plasma cleaner (PDC-32G, Harrick Plasma, Ithaca, NY) for 8 min at a media setting. Polydopamine was coated on fiber membranes, according to previous studies [5,26]. Briefly, fiber membranes were immersed in either 0.2 or 2 mg ml^{-1} dopamine·HCl aqueous solution to prepare a thin and regular coating of PCL fibers. Then, the pH value of solution was adjusted up to 8.5. The processes took 4 h and 24 h for thin and regular coating, respectively. The polymerizing solution was replaced with fresh solution at 12 h in the latter case. Polydopamine-coated fibers were then washed with DI water to remove excess monomer and particles.

2.2. Morphological characterization

The morphology and structure of solvent-extracted polydopamine-coated fiber samples were characterized by scanning electron microscopy (SEM; FEI, Nova2300, Oregon). To avoid charging, polymer fiber samples were fixed on a metallic stud with double-sided conductive tape and coated with platinum for 40 s in vacuum at a current intensity of 40 mA, using a sputter coater. SEM images were acquired at an accelerating voltage of 15 kV. Transmission electron microscopy (TEM; FEI Spirit) was further used to acquire images of solvent-extracted samples, which were mounted on carbon-coated copper grids.

2.3. In vitro loading kinetics

The loading kinetics of model drugs (R6G and DOX) to fibers in aqueous solutions were tested using air-plasma-treated-PCL fibers, air-plasma-treated-PCL fibers with thin polydopamine coating and air-plasma-treated-PCL fibers with regular polydopamine coating. R6G aqueous solutions (3.3 μg ml^{-1}) were prepared with pH values of 2.0, 5.0, 7.0, 9.0 and 11.0. Similarly, DOX aqueous solutions (5 μg ml^{-1}) were prepared with pH values of 2.0, 5.0, 7.0 and 9.0. For tests of loading kinetics for both R6G and DOX, ~5-mg fiber samples were put into a tube, and then 2 ml prepared drug solution was added. Fiber samples were immersed in the solution, and all the tubes were placed on a rocker (30 rpm) at 25 °C. At each time point, 20 μl supernatant was collected and then diluted 10 times and placed in the wells of a 96-well plate. After the solution was collected, 20 μl water with corresponding pH value was added to the scintillation tube in order to keep the total volume constant at 2 ml. Both R6G and DOX supernatants were collected every 3 min for 18 min in total. In addition, DOX supernatants were collected at 12 h and 24 h for tests of loading kinetics. A micro-plate reader was then used to measure the fluorescent intensity of each sample collected at an excitation wavelength of 480 nm and an emission wavelength of 590 nm. Morphologies of PCL fibers with regular polydopamine coatings after incubation in the 3.3 μg ml^{-1} R6G solution at pH 2.0, 5.0, 7.0 and 9.0 for 20 min were observed under a fluorescence microscope (AX10, Zeiss). Fluorescence microscopy (FM) images were taken with the same exposure time. Optical microscopy (OM) images were also taken in the same visual field.

2.4. In vitro release

In vitro release of R6G and DOX from the fibers was evaluated using drug-loaded nanofiber membranes. The polydopamine-coated nanofiber membranes (~30 mg) were incubated with