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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 pH values at specific time as per the pathophysiological need of the disease. Herein, we demonstrated 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 showed that the positive charged molecules released 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 (MTT) assays showed the viability of cancer cells after treatment with doxorubicin released media at different pH values qualitatively and quantitatively, indicating the media contained doxorubicin which was released in solutions at low pH values could kill significantly higher number of cells than that released in solutions at high pH values. Together, the pH-responsive drug delivery systems based on polydopamine-coated PCL nanofibers could have potential applications in oral delivery of anticancer drugs for treating gastric cancer and 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.

Keywords

pH-sensitive; Drug delivery; Polydopamine coating; Poly (ϵ -caprolactone); Electrospun fibers

1. Introduction

Controlled drug delivery systems (CDDS) have been widely investigated for treating many diseases [1, 2]. Normally, CDDS are developed based on two criteria. One is the temporal drug modulation matching the physiological needs and the other is the drug distribution on a specific target [3]. Nano-scale materials are coming into notice 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 its 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 to electrospun fibers for controlled release including small molecular drugs, herbs, peptides, proteins, DNA and vaccines [7, 8]. Wnek *et al.* first reported electrospun fiber mats made either 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 mainly controlled by diffusion due to slow degradation of PLA and non-degradable property of PEVA. The 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) [9]. 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 time as per the pathophysiological need of the disease, stimuli-responsive drug delivery is very critical. The stimuli-responsive drug delivery systems are in the vanguard of drug administration as it could response to small signs and changes in the surrounding environment, which translates into significant changes in their microstructure and in the physiological and chemical proprieties, as desired [14,15]. The signs or stimuli that trigger the structural changes on these smart polymers can be classified in three main categories: physical stimuli (i.e., temperature, ultrasounds, light and mechanical stress), chemical stimuli (i.e., pH and ionic strength) and biological stimuli (i.e., enzymes and bio-molecules) [3]. Due to the remarkable changes of pH value in human body, pH-responsive drug delivery systems have been designed for carrying and directing therapeutic agents to a specific body area, tissue or organ. The representative examples may 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]. However, so far few studies have examined the pH-responsive drug delivery systems composed of electrospun nanofibers which could be due to the difficulty of fabrication of such smart fibers using electrospinning technique. Zhai *et al.* fabricated methylene blue-loaded ultrathin fibers comprising PAA and poly (allylamine hydrochloride) (PAH) and demonstrated the controlled release of methylene blue (low molecular weight cationic molecules) from fibers by pH in a nonbuffered solutions based on the replacement of protons in carboxylate groups in fibers [18]. In another study, Li *et al.* developed electrospun nanofibers of acid-labile biodegradable polymers containing ortho ester groups or acetal groups for pH controlled release of paracetamol [19, 20]. In a separate study, De Smedt *et al.* demonstrated the incorporation of anti-viral drugs in electrospun cellulose acetate phthalate fibers and the fibers are stable in healthy vaginal fluid (pH < 4.5) and become dissolvable immediately upon addition of small amount of human semen (pH between 7.4 and 8.4) which causes the release of encapsulated drugs, indicating the potential for prevention of human immunodeficiency virus transmission [21].

We have recently reported the polydopamine-mediated surface modification to electrospun poly (ϵ -caprolactone) (PCL) and poly (lactic acid) (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 high reactive carbonyl (-CO-), carboxyl (-COOH), and hydroxyl (-OH) groups after oxygen containing plasma treatment [22–24]. Further, recent studies demonstrated 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. We hypothesized that the release of charged molecules from air-plasma treated electrospun PCL nanofibers could be responsive to different pH values. We 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 solution's pH value. MTT assay of H1299 was conducted by using DOX releasing cell culture medium, indicating at low pH value DOX releasing medium could kill more cancer cells than high pH value. It revealed that polydopamine-coated PCL nanofibers may have potential applications in oral and topical drug delivery to the targets where is related to the changes of pH values.

2. Materials and methods

2.1. Fabrication of electrospun fibers and polydopamine coating

Fibers were produced utilizing a standard electrospinning setup as described earlier [5], PCL (Mw= 80 kDa, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in a solvent mixture consisting of dichloromethane (DCM, fisher chemical, Waltham, MA, USA) and N,N-dimethylformamide (DMF; fisher chemical, Waltham, MA, USA) with 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 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 less than 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 dopamine-HCl aqueous solution for preparing 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 the fresh one at 12 h for 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. TEM (FEI Spirit) was further utilized to acquire images of solvent-extracted samples, which were mounted on carbon-coated copper grids.

2.3. In vitro loading kinetics

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) were prepared with pH values of 2.0, 5.0, 7.0, 9.0 and 11.0. Similarly, DOX aqueous solutions (5 µg/mL) were prepared with pH values of 2.0, 5.0, 7.0 and 9.0. For tests of loading kinetics of both R6G and DOX, around 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. 20 μ L supernatant was collected at each time point and then diluted 10 times and placed into wells of a 96-well plate. After the solution was collected, 20 μ L 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 also 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 μ g/mL R6G solution at pH of 2.0, 5.0, 7.0 and 9.0 for 20 min were observed under fluorescence microscope (AX10, Zeiss). Fluorescence images (FM) were taken with the same exposure time. Optical images (OM) 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 ($\approx$ 30 mg) were incubated with 30 mL drug solutions (R6G 3.3 μ g/mL or DOX 5 μ g/mL) at pH 9.0 for 12 h. Then the fiber membrane was rinsed in 20 mL deionized water (pH 9.0) for three times. All the solutions after incubation and rinsing were collected and the concentration was determined by the method described in section 2.3 which was used to calculate total drug loaded. Around 5 mg R6G-loaded fiber samples were immersed in water at different pH values (2.0, 5.0, 7.0, 9.0 and 11.0). Similarly, 5 mg DOX-loaded fibers samples were soaked in water at various pH values (2.0, 5.0, 7.0 and 9.0). The samples were kept on a rocker (30 rpm) at constant temperature of 25.0 °C. At given time intervals, the solution was withdrawn and kept for analysis as described in section 2.3.

2.5. Cell culture

H1299 cells were maintained in a complete media made of Dulbecco's Modified Eagle Media (D-MEM) with 10% Fetal Calf Serum. The cultures were maintained at 37 °C under 95% air and 5% CO₂. The subconfluent monolayers were dissociated with a 0.01% solution of trypsin and resuspended into a fresh complete media. The culture media for DOX release from fiber materials is D-MEM and the pH value was adjusted to 2.0, 5.0, 7.0 and 9.0 using hydrochloric acid or sodium hydroxide solution, respectively. After collection of releasing media, the pH value was re-adjusted back to 7.2–7.4. Then, the releasing media was sterilized by 0.22 μ m filters and incubated with cells for different periods of time.

2.6. Fluorescein diacetate staining and MTT assay

Fluorescein diacetate (FDA) stock solution was prepared by dissolving 10 mg of FDA in 1 mL acetone. Cells were placed in 24-well culture plates (1×10^4 cells for 24 h treatment and 5×10^3 for 72 h treatment per well). The viable cells after treating with DOX released media at different incubation times was stained with 20 μ g/mL FDA working solution (diluted stock solution in PBS) for 3 min and then observed by fluorescence microscopy. Standard 3-(4, 5-dimethylthiazole)-2, 5-diphenyltetrazolium bromide (MTT) assay procedures were used to quantify the cell viability after treatment with DOX-released media. Cells were placed in 96-wells microassay culture plates (5×10^3 cells for 24 h treatment and 2.5×10^3 for 72 h treatment per well) and incubated in a 95% air and 5% CO₂ incubator for overnight at 37 °C. The original media was replaced by the DOX released media (150 μ L). The basic culture media were added to microwells which were taken as negative controls. The culture plates were incubated at 37 °C in a 5% CO₂ incubator for 1 day and 3 days. Upon completion of the incubation, stock MTT assay prepared in phosphate buffer solution (PBS) (10 μ L, 5 mg/mL) was added to each well. After incubation for 3 h, media was replaced by

100 μ L dimethyl sulfoxide to solubilize the MTT formazan. The optical density of each well was then measured using a micro-plate spectrophotometer at a wavelength of 490 nm. The cell viability was determined by the percentage of number of cells treated by DOX released solutions relative to the control. Each experiment was repeated at least five times.

2.7. Statistical analysis

Three replicates were tested for each data point. The statistical analysis was performed on the means of the data obtained from at least three independent experiments. All the results were presented as means and the significance was assessed using student's t-test. *p* values of 0.05–0.01 and 0.01 or less among the groups were considered to be significant and very significant, respectively.

3. Results

3.1. Fabrication of polydopamine-coated PCL fibers

PCL nanofibers were first fabricated by electrospinning and then treated with air plasma. Subsequently, polydopamine coating on PCL fibers was performed based on our recent study [5]. Fig. 1A shows a SEM image of polydopamine tubes which were obtained by soaking the PCL-polydopamine core-sheath nanofibers in DCM to selectively remove the cores. Fig. 1B shows a TEM image of the same samples shown in Fig. 1A, indicating the presence of polydopamine coating and the thickness of surface coating was around 20 nm. In addition, both SEM and TEM images suggested the diameter of polydopamine-coated PCL nanofibers was around 250 nm.

3.2. In vitro loading and releasing kinetics of R6G

We chose R6G as model molecules for loading and releasing kinetic studies as it is positively charged and easily detected. Prior to examination of releasing kinetics of R6G, we first investigated the loading kinetics of R6G into various fiber samples including pristine PCL nanofibers, PCL nanofibers with thin polydopamine coating (0.2 mg/mL dopamine was polymerized for 4 h at pH 8.5), and PCL fibers with regular polydopamine coating (2 mg/mL dopamine was polymerized for 12 h at pH 8.5 and this procedure was repeated once) in aqueous solutions (3.3 μ g/mL) at pH values of 2.0 and 9.0 (Fig. 2A). It seems that the loading capacities of R6G could become lower with increasing the thickness of polydopamine coating when the pH value was 2.0. In contrast, the loading kinetics of R6G had marginal differences among the samples when the pH value was 9.0. In addition, it is noticed that the R6G loading capacities were significantly higher in solutions with higher pH values. It is worth noting that air plasma treated PCL nanofibers presented significant differences of R6G loading capacity and release profiles in acidic and basic solutions. The R6G loading kinetics and capacity in aqueous solutions at pH 9.0 were comparable between PCL nanofibers with thin polydopamine coating and uncoated fibers. In contrast, in aqueous solutions at pH 2.0 the coating thickness seemed to inhibit the uptake of R6G to certain extent. Based on the calculations, the total amount of R6G loaded to pristine PCL nanofibers, PCL nanofibers with thin polydopamine coating, and PCL fibers with regular polydopamine coating were 532 ± 40 ng, 551 ± 59 ng, and 567 ± 55 ng per mg fiber samples at pH 9.0.

The samples for *in vitro* release study were loaded with drugs at the same condition, meaning that the samples had the same drug loadings. When we performed the drug release study, the samples were immersed in the solutions with different pH values. Specifically, the fiber samples for R6G cumulative release was firstly taken up in R6G solution at pH 9.0 and rinsed in water at pH 9.0 to remove loosely attached molecules on the fiber surface. Subsequently, we examined the *in vitro* release profiles of R6G from fiber samples (Fig.

2B). It is observed that nearly 80% R6G loaded was released from PCL nanofibers without and with coating thin layer of polydopamine at first 3 min and almost all R6G was released in 20 min in solutions at pH 2.0. Accordingly, around 20% and 60% of R6G were released respectively from PCL nanofibers with regular polydopamine coating within 3 min and 20 min in solutions at pH 2.0. Contrarily, the releasing rates for all the fiber samples were much slower in solutions at pH 9.0. The corresponding cumulative release for pristine PCL nanofibers, PCL nanofibers with thin polydopamine coating, and PCL fibers with regular polydopamine coating were 50.4%, 35.5% and 13.0% at pH 9.0. PLA nanofibers also showed the similar property (Fig. S1).

In order to better understand the influence of pH, we further examined the loading and releasing kinetics of R6G from PCL fibers with regular polydopamine coating at pH values of 2.0, 5.0, 7.0, 9.0 and 11.0 (Fig. 3). It is seen that the loading capacity increased with increasing pH value (Fig. 3A). Accordingly, R6G released slowly at pH 7.0, 9.0 and 11.0, but much faster at pH 5.0 and 2.0 on the contrary (Fig. 3B). The fluorescence microscopy images showed the increase of fluorescent intensity with increasing pH values, further demonstrating the difference of R6G loading under different pH values (Fig. 4).

3.3. Loading kinetics and in vitro release of doxorubicin

To further demonstrate the proof-of-concept, we chose DOX as a model drug for investigating its loading kinetics and in vitro release to and from PCL nanofibers with regular polydopamine coating in solutions at pH 2.0, 5.0, 7.0 and 9.0 (Fig. 5). Similarly to R6G, the results showed that the DOX loading capacity of PCL nanofibers with regular polydopamine coating increased with increasing pH values of the releasing solutions (Fig. 5A). Based on the calculations, the total amount of DOX loaded to polydopamine coated PCL nanofibers which were used in releasing tests was 2.3 ± 0.2 μg per mg fiber samples at pH 9.0. It is seen that the in vitro release rate of DOX was very slow at pH values of 7.0 and 9.0 and the amount of released DOX was less than 10% of total drug loading during the releasing period of 12 h (Fig. 5B). By contrast, the in vitro release rate of DOX from polydopamine-coated PCL fiber samples was much higher at pH values of 2.0 and 5.0 compared to 7.0 and 9.0 and around 79.9% and 50.2% of loaded drugs were released in solutions at pH values of 2.0 and 5.0 within the same incubation period (Fig. 5B).

3.4. Cell viability

To further prove the pH-responsive drug delivery based on polydopamine-coated PCL nanofibers, we examined the cell viability after treatment with the DOX released media using H1299 cell line as model cancer cells. The in vitro release of DOX from polydopamine-coated PCL nanofibers was firstly carried out in the cell culture media (DMEM) where the pH values were adjusted to 2.0, 5.0, 7.0 and 9.0. To avoid the influences of pH values on cell viability, the pH values of all DOX released media were re-adjusted back to 7.2–7.4 prior to incubation with cells. The DOX released media were collected after incubation for 3 min, 20 min, and 120 min. To qualitatively illustrate the viability of cancer cells, we stained the live cells with FDA (a cell-permeant esterase substrate serving as a viability probe that measures both enzymatic activity and cell-membrane integrity) in green after incubation with DOX released media for 1 day and 3 days [27]. Fig. 6 showed fluorescence images of live cells stained with FDA after administration of DOX released media for 1 day and 3 days, respectively. With increasing pH values, more cells can survive as less amount of DOX was released out. Fewest cells survived when the cells were treated with DOX released media which was collected at 120 min for 3 days. We further used MTT assay to quantify the cell viability after administration of DOX released media for 1 day and 3 days (Fig. 7). The cell viability was significantly lower when treating DOX released media in the acidic environment (pH: 2.0 and 5.0) compared to the neutral and basic environments

(pH: 7.0 and 9.0). In addition, the cell viability was the lowest when administrating with DOX released media collected in the acidic environment at 120 min compared to 3 min and 20 min. Both incubation times (1 day and 3 days) showed the similar trend. The results of cytotoxicity test further confirmed the pH-responsive drug release from polydopamine-coated PCL nanofibers.

4. Discussion

The variations in pH values have been found in some endosomal compartments of cells and different tissues or organs (i.e., tumor tissue, inflamed tissue, stomach, vaginal fluid) [28]. A notable example is gastrointestinal system. The pH value in stomach is around 1.0–3.0. After the acid is neutralized by gland secretions of pancreatic, biliary and Brunner's in duodenum, the pH value can turn into 4.8–8.2 and then become stable ranging from 7.0 to 7.5 in colon [3, 28, 29]. Recently, electrospun nanofibers show great potential as implantable drug carriers and functional coating of medical devices for local drug delivery [30, 31]. Use of electrospun nanofibers for pH-responsive drug delivery to particular targets could be an intriguing approach. According to this study, polydopamine-coated PCL nanofibers could release drugs in the acidic aqueous solution rather than in neutral and basic aqueous solutions. Such fiber materials may have potential applications as drug delivery carrier for oral chemotherapy to treat gastric cancer. Oral pills made up of polydopamine coated PCL nanofibers could carry drugs to stomach and then release them near nidus very quickly. When these pills go along the digestive tract into duodenum and colon, the drug release rate may significantly decrease. Administering such formulations may raise the efficacy of oral chemotherapeutic drugs and minimize toxic side effects and thus eventually improve patients' life. The most commonly used dosage of doxorubicin is around 20 mg/m² every two weeks for chemotherapy of usual adult [32]. In order to deliver this dose of drugs, only 10 g or less DOX-loaded fibers are needed around every 14 days.

Based on the results of loading kinetics and in vitro release profiles, air plasma-treated PCL nanofibers exhibited pH responsive properties (Figs. 2 and 3). The similar results were also observed for air plasma-treated PLA nanofibers (Fig. S1). Air plasma treatment has been utilized to improve surface hydrophilicity and develop functional groups in a variety of polymeric biomaterials [24]. Previous studies suggested that weak-chemical bonds present in PCL were replaced by highly reactive groups including carbonyl (-CO-), carboxyl (-COOH), and hydroxyl (-OH), resulting in profound biochemical activation of the material following treatment [22, 33]. In this work, air plasma treatment to electrospun PCL and PLA nanofibers was found to increase hydrophilicity and exhibit superior wettability. The carboxyl (-COOH) groups increased on the surface of air plasma-treated fibers which could also respond to pH resulting in protonation or deprotonation. This may explain the reason why the increased pH caused higher loading capacity for air plasma-treated PCL fibers, which is line with the mechanism of many pH-responsive drug delivery systems.

Dopamine can be polymerized in basic solution to form polydopamine coating on many substrates including metals, organic and inorganic materials [26]. In this study, we demonstrated polydopamine coating on the surface of air plasma-treated, electrospun PCL nanofibers. It seems that polydopamine coating could act as a mediator for enhancing the pH selective loading in aqueous solutions. Most importantly, polydopamine coating can finely tune the pH-sensitive drug release from electrospun nanofibers. We have proposed the following speculations. The polydopamine coating on the nanofibers could be divided into two steps. At the beginning, oxidation and rearrangement of dopamine could occur in aqueous solutions at pH 8.5, resulting in the resultant 5, 6-dihydroxyindole which is the monomer for polymerization in the following step [34]. Cyclization of amino group (-NH₂) could take place and hydroxyl (-OH) groups could remain for the polymer after this reaction

[26, 35]. It is well known that hydroxyl group containing compounds always liberate hydrogen cations and make the parent compound an acid [33]. Air plasma treatment to electrospun PCL nanofibers can generate active functional groups (i.e., -CO-, -COOH) which could help induce the occurrence of dopamine polymerization on the surface of fibers and further promote the formation of an even coating of polydopamine [5]. pH sensitive polymers named as polyacids or polyanions responded to variations of pH as they have a great number of functional groups such as carboxylic acid or sulfonic acid groups in their molecular structures [3]. Modified surfaces of nanofibers which have hydroxyl groups may also present the similar property. It could release hydrogen cations and create negative charges on the surface in neutral and basic environments. Correspondingly, hydroxyl groups could be protonated and the surface could become electrically neutral in acid [34].

One-dimensional drug diffusion model seems not suitable to fit the actual release rates of DOX from PDPCL nanofibers (Fig. S2). A desorption model reported as follows was used to describe the release of hydrophilic dye from nanofibers in aqueous solution [35].

$$\frac{M_t}{M_\infty} = \alpha \left[1 - \exp\left(-\frac{\pi^2}{8} \frac{t}{\tau_r}\right) \right] \quad (1)$$

Where M_t is the mass of drug released at time t , M_∞ is the mass of drug released as time approaches infinity (or equivalently the total amount of drug encapsulated), α is nanoporosity τ_r factor and τ_r is characteristic time. The in vitro release data for DOX from doxorubicin-loaded PDPCL fibers was fitted with Eq. (1), thus giving

$$\frac{M_t}{M_\infty} = 0.74 \left[1 - \exp\left(-\frac{\pi^2}{8} \frac{t}{0.24}\right) \right] \quad r^2 = 0.988 \quad (2)$$

For doxorubicin-loaded PDPCL fibers releasing kinetics at pH 2.0

$$\frac{M_t}{M_\infty} = 0.46 \left[1 - \exp\left(-\frac{\pi^2}{8} \frac{t}{0.22}\right) \right] \quad r^2 = 0.982 \quad (3)$$

For doxorubicin-loaded PDPCL fibers releasing kinetics at pH 5.0

$$\frac{M_t}{M_\infty} = 0.09 \left[1 - \exp\left(-\frac{\pi^2}{8} \frac{t}{0.51}\right) \right] \quad r^2 = 0.983 \quad (4)$$

For doxorubicin-loaded PDPCL fibers releasing kinetics at pH 7.0

$$\frac{M_t}{M_\infty} = 0.08 \left[1 - \exp\left(-\frac{\pi^2}{8} \frac{t}{0.47}\right) \right] \quad r^2 = 0.978 \quad (5)$$

For doxorubicin-loaded PDPCL fibers releasing kinetics at pH 9.0 Fig. 5 B plotted the experimental data and the curves after fitting to equation 1 with an appropriate choice of α and τ_r . It seems that the release data can fit perfectly with this desorption model, indicating that desorption could be the limiting step for the DOX release from PDPCL nanofibers in the aqueous solutions with different pH values.

R6G has been employed as a model drug in order to investigate drug releasing kinetics of delivery system [36]. DOX is commonly used to treat some leukemia and Hodgkin's lymphoma, as well as cancers of the bladder, breast, stomach, lung, ovaries and others [36, 37]. It interacts with DNA by intercalation and inhibition of macromolecular biosynthesis and combats tumors via a mechanism called regulated intramembrane proteolysis [38]. The released doxorubicin from nanofibers should interact with normal cells in a similar mechanism. In this study, the polydopamine-coated fibers were applied as a doxorubicin delivery vehicle to raise local drug concentration near nidus and limit the release of drugs around normal tissues. Therefore, the nanofiber-based delivery systems are expected to raise the efficacy of oral chemotherapeutic drugs and minimize toxic side effects. In addition, Harrington *et al.* reported the discovery of dopa-Fe bond [26]. Both DOX and R6G are hydrochloride salts which have positive charges after solvating in water. Such drugs could be electrostatically attracted by the negative charged surface. As electrospun nanofibers have high surface area-to-volume ratio and a large number of hydroxyl groups have been created due to polydopamine coating on the surface, cations could be captured by polydopamine-coated PCL nanofibers. The hydroxyl groups on the surface tended to protonate when the pH value was decreased. Thus, the negative charges on the surface of fibers dribbled away, which could cause the release of DOX and R6G from polydopamine-coated PCL nanofibers.

Our previous study demonstrated that polydopamine coated PCL fibers are non-toxic and biocompatible and fibroblasts can adhere and proliferate well on such fibers [5]. Cytotoxicity test showed that cell viability dramatically decreased when cells were treated with DOX released solutions collected at lower pH. In addition, longer releasing time resulted in more DOX in media which caused significant decrease of cell viability. Here, the release kinetics in water and DMEM culture media were assumed the same. The cytotoxicity results further confirmed pH-responsive release of DOX from polydopamine-coated PCL nanofibers and toxicology data which was reported by Rathos *et al.* using propidium iodide assay [39].

5. Conclusions

In this study, we demonstrated that air plasma-treated, electrospun PCL and PLA nanofibers can be used as carriers for loading and release of charged molecules in a pH-responsive way. We also demonstrated that a mussel-inspired protein polydopamine coating can finely tailor the pH-responsive loading kinetics and release of charged molecules. These novel formulations could have potential applications in drug delivery to specific targets which are related to pH variations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. (A) SEM image showing polydopamine tubes. (B) TEM image showing the same sample in (A). The inset: magnified image of (B). The tubes were obtained by soaking the PCL-polydopamine core-sheath nanofibers in DCM to selectively remove the cores. The polymerization was conducted in the 2.0 mg/mL dopamine solution at pH=8.5 for 12 h and this coating procedure was repeated once.

Fig. 2. Rhodamine 6G loading kinetics (A) and release profiles (B) of various samples in aqueous solutions at pH of 2.0 and 9.0. Square (uncoated): air plasma treated PCL fibers. Triangle (thin): air plasma treated PCL fibers were coated with polydopamine in 0.2 mg/mL dopamine solution for 4 h. Circle (coated): air plasma treated PCL fibers were coated with polydopamine in 2.0 mg/mL dopamine solution for 12 h and this coating procedure was repeated once. Each data point shown is the average of three samples.

Fig. 3. Rhodamine 6G loading kinetics (A) and release profiles (B) of polydopamine coated PCL fiber samples in aqueous solutions at pH values of 2.0, 5.0, 7.0, 9.0 and 11.0. The samples were first treated with air plasma and thus coated with polydopamine in 2.0 mg/mL dopamine solution for 12 h and this coating procedure was repeated once. Each data point shown is the average of three samples.

Fig. 4. Fluorescence (FM) and optical microscopy (OM) images of polydopamine-coated PCL fiber samples which were immersed in 2.5 $\mu\text{g/mL}$ rhodamine 6G aqueous solution for 20min at pH values of 2.0, 5.0, 7.0, and 9.0. The samples were first treated with air plasma and thus coated in 2.0 mg/mL dopamine for 12 h and this coating procedure was repeated once. The scale bar in the image is 50 μm .

Fig. 5.

Doxorubicin release profiles of polydopamine-coated PCL fiber samples in aqueous solutions at pH of 2.0, 5.0, 7.0, and 9.0. The samples were first treated with air plasma and thus coated in 2.0 mg/mL dopamine for 12 h and this coating procedure was repeated once. The in vitro release data was fitted with a desorption model. L2.0: $\alpha = 0.74$, $\tau_r = 0.24$, L5.0: $\alpha = 0.46$, $\tau_r = 0.22$, L7.0: $\alpha = 0.09$, $\tau_r = 0.51$, L9.0: $\alpha = 0.08$, $\tau_r = 0.47$.

Fig. 6. Fluorescence microscopy images of H1299 cells (stained with fluorescein diacetate in green) after incubation with the released media for 1 day (A) and 3 days (B). The released media from doxorubicin loaded, polydopamine-coated PCL fiber samples were collected at different times (3 min, 20 min, and 120 min) from solutions with pH values of 2.0, 5.0, 7.0 and 9.0. The scale bar in the image is 50 μ m.

Fig. 7. H1299 cell viability quantified by MTT assay. The cells were treated with the released media for 1 day (A) and 3 days (B). The released media from doxorubicin loaded, polydopamine-coated PCL fiber samples were collected at different times (3 min, 20 min, and 120 min) from solutions with pH values of 2.0, 5.0, 7.0 and 9.0. (* $p < 0.05$, ** $p < 0.01$)