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

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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(e-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.

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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 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(e-caprolactone) (PCL) fiber meshes with the hydrophobic polymer dopant poly(glycerol monostearate-co-e-caprolactone) (PGC-C18) for camptothecin-11 or irino-tecan 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 carbonyl (−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 samples was conducted using a DOX-releasing medium to carry out the experiment, 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 electrosprinning setup as described earlier [5], PCL (Mw = 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 (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 microplate reader was then used to measure the fluorescence 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
30 ml drug solutions (R6G \(3.3 \mu g \text{ml}^{-1}\) or DOX \(5 \mu g \text{ml}^{-1}\)) at pH 9.0 for 12 h. Then the fiber membrane was rinsed three times in 20 ml deionized water (pH 9.0). 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 the total drug loaded. R6G-loaded fiber samples of \(~5 \text{ mg}\) 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 a 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 complete media made of Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal calf serum. The cultures were maintained at 37°C under 95% air and 5% CO\(_2\). 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 was DMEM, 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 readjusted back to 7.2–7.4. Then, the releasing media was sterilized by 0.22 μm 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 treatment with DOX-released media at different incubation times was stained with 20 μg ml\(^{-1}\) FDA working solution (diluted stock solution in phosphate buffer solution (PBS)) for 3 min and then observed by fluorescence microscopy. Standard MTT assay procedures were used to quantify the cell viability after treatment with DOX-released media. Cells were placed in 96-well 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% CO\(_2\) incubator overnight at 37°C. The original media was replaced by the DOX released media (150μl). The basic culture media were added to the microwells, which were taken as negative controls. The culture plates were incubated at 37°C in a 5% CO\(_2\) incubator for 1 day and 3 days. Upon completion of the incubation, stock MTT assay prepared in PBS (10 μl, 5 mg ml\(^{-1}\)) was added to each well. After incubation for 3 h, the medium was replaced by 100 μl 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. The 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 a recent study [5]. Fig. 1A shows a SEM image of polydopamine tubes 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 sample shown in Fig. 1A, indicating the presence of polydopamine coating, and the thickness of surface coating was \(~20\) nm. In addition, both SEM and TEM images suggest that the diameter of PDPCL nanofibers was \(~250\) nm.

3.2. In vitro loading and releasing kinetics of R6G

R6G was chosen as the model molecule for loading and releasing kinetic studies, as it is positively charged and easily detected. Prior to examination of releasing kinetics of R6G, the loading kinetics of R6G into various fiber samples was first investigated, including pristine PCL nanofibers, PCL nanofibers with thin polydopamine coating (0.2 mg ml\(^{-1}\) dopamine was polymerized...
for 4 h at pH 8.5), and PCL fibers with regular polydopamine coating (2 mg ml\(^{-1}\) dopamine was polymerized for 12 h at pH 8.5 and this procedure was repeated once) in aqueous solutions (3.3 mg ml\(^{-1}\) dopamine was polymerized for 12 h at pH 8.5 and this procedure was repeated once) in aqueous solutions (3.3 mg ml\(^{-1}\) at pH values of 2.0 and 9.0 (Fig. 2A). It seems that the loading capacities of R6G could become lower with increasing 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 in 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 a certain extent. Based on the calculations, the total amounts 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 mg\(^{-1}\) fiber samples at pH 9.0.

The samples for in vitro release study were loaded with drugs in the same condition, meaning that the samples had the same drug loadings. When the drug-release study was performed, the samples were immersed in the solutions with different pH values. Specifically, the fiber samples for R6G cumulative release were first 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, the in vitro release profiles of R6G from fiber samples were examined (Fig. 2B). It was observed that nearly 80% R6G loaded was released from PCL nanofibers without and with a thin layer coating of polydopamine at the first 3 min, and almost all R6G was released in 20 min in solutions at pH 2.0. Accordingly, ~20% and 60% of R6G were released from PCL nanofibers with regular polydopamine coating within 3 min and 20 min, respectively, in solutions at pH 2.0. In contrast, 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 a similar property (Fig. 5).

In order to understand the influence of pH better, the present authors 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). 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 in contrast (Fig. 3B). The FM images show the increase in fluorescent intensity with increasing pH values, further demonstrating the difference in R6G loading under different pH values (Fig. 4).

### 3.3. Loading kinetics and in vitro release of doxorubicin

To demonstrate the proof of concept further, DOX was chosen as a model drug for investigating its loading kinetics and in vitro release to and from PCL nanofibers with regular polydopamine coating.
coating in solutions at pH 2.0, 5.0, 7.0 and 9.0 (Fig. 5). Similarly to R6G, the results show 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 PDPCL nanofibers that were used in releasing tests was $2.3 \pm 0.2 \mu g mg^{-1} fiber samples$ at pH 9.0. 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 <10% of total drug loading during the releasing period of 12 h (Fig. 5B). By contrast, the in vitro release rate of DOX from PDPCL fiber samples was much higher at pH values of 2.0 and 5.0 compared with 7.0 and 9.0, and ~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 PDPCL nanofibers, the cell viability after treatment with the DOX-released media was examined using the H1299 cell line as model cancer cells. The in vitro release of DOX from PDPCL nanofibers was first 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 readjusted 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 illustrate the viability of cancer cells qualitatively, the live cells were stained 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 shows 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 DOX is released out. Fewest cells survived when the cells were treated with DOX-released media collected at 120 min for 3 days. MTT assay was further used 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 an acidic environment (pH 2.0).
and 5.0) compared with the neutral and basic environments (pH 7.0 and 9.0). In addition, the cell viability was the lowest when administered with DOX-released media collected in the acidic environment at 120 min compared with 3 min and 20 min. Both incubation times (1 day and 3 days) showed a similar trend. The results of the cytotoxicity test further confirmed the pH-responsive drug release from PDPCL nanofibers.

4. Discussion

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 the gastrointestinal system. The pH value in the stomach is ~1.0–3.0. After the acid is neutralized by gland secretions of pancreatic, biliary and Brunner's in the duodenum, the pH value can turn into 4.8–8.2 and then become stable, ranging from 7.0 to 7.5 in the colon [3,28,29]. Recently, electrospun 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 why the increased pH caused a higher loading capacity for air-plasma-treated PCL fibers, which is in 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]. This study 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 pH-sensitive drug release from electrospun nanofibers. The following speculations are proposed. 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 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 the 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 called polyacids or polyanions respond to variations in 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 that have hydroxyl groups may also present a 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].

The one-dimensional drug diffusion model does not seem 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]:

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

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

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

for doxorubicin-loaded PDPCL fibers releasing kinetics at pH 2.0

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

for doxorubicin-loaded PDPCL fibers releasing kinetics at pH 5.0

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

for doxorubicin-loaded PDPCL fibers releasing kinetics at pH 7.0

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

for doxorubicin-loaded PDPCL fibers releasing kinetics at pH 9.0.

Fig. 5B plots the experimental data and the curves after fitting to Eq. (1) with an appropriate choice of $\alpha$ and $\tau_r$. It seems that the release data fit perfectly with this desorption model, indicating that desorption could be the limiting step for the DOX release from PDPCL nanofibers in aqueous solutions with different pH values.

R6G has been employed as a model drug in order to investigate the drug-releasing kinetics of delivery systems [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 doxorubicin released 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 the 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 the dopa–Fe bond [39]. Both DOX and R6G are hydrochloride salts that have positive charges after solvating in water. Such drugs could be electrostastically attracted by the negative charged surface. As electrospun nanofibers have a high surface-area-to-volume ratio and a large number of hydroxyl groups have been created as a result of polydopamine coating on the surface, cations could be captured by PDPCL 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.

A previous study demonstrated that PDPCl fibers are non-toxic and biocompatible, and fibroblasts can adhere and proliferate well on such fibers [5]. A cytotoxicity test showed that cell viability dramatically decreased when cells were treated with DOX-released solutions collected at lower pH. In addition, a longer releasing time resulted in more DOX in the media, which caused a significant decrease in cell viability. Here, the release kinetics in water and DMEM culture media were assumed to be the same. The cytotoxicity results further confirmed pH-responsive release of DOX from PDPCL nanofibers and toxicity data, which was reported by Rathos et al. using propidium iodide assay [40].

5. Conclusions

This study 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. It 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 application in drug delivery to specific targets that are related to variations in pH.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actbio.2013.11.012.

Appendix B. Figures with essential colour discrimination

Certain figures in this article, particularly Figs. 4 and 6 are difficult to interpret in black and white. The full colour images can be found in the on-line version, at http://dx.doi.org/10.1016/j.actbio.2013.11.012.

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