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Research paper

Electrospun fibers of acid-labile biodegradable polymers containing ortho ester groups for controlled release of paracetamol

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ABSTRACT

The local delivery and controllable release profiles make electrospun ultrafine fibers as potential implantable drug carriers and functional coatings of medical devices. There are few attempts to form acid-labile electrospun fibers, whose release behaviors respond to the local environment and fiber characteristics. In the current study a novel strategy was presented to synthesize biodegradable pH-sensitive polymers containing ortho ester groups. The acid-labile segments were synthesized through reacting 3,9-dimethylene-2,4,8,10-tetraoxaspiro [5.5] undecane with 1,10-decanediol or poly(ethylene glycol), which were further copolymerized with D,L-lactide to obtain triblock copolymers. Biodegradable acid-labile polymers were electrospun with the encapsulation of paracetamol as a model drug. *In vitro* release study showed that the total amount of drug released from acid-labile polymeric fibers was accelerated after incubation into acid buffer solutions, and the amount of initial burst release and sustained release rate were significantly higher for matrix polymers with hydrophilic acid-labile segments. *In vitro* degradation study indicated that the electrospun fibers containing acid-labile segments were stable in neutral buffer solution, but the molecular weight reduction of matrix polymers, the morphological changes and mass loss of fibrous mats were significantly enhanced under acid circumstances.

1. Introduction

In recent years, research and development in the field of drug delivery systems facilitating site-specific therapy has achieved significant progression. Site-specific delivery and specific activation of the biologically active compound at specific organs, tissues and cells have become the major goal of such research. A local therapy has many potential advantages such as circumventing possible adverse side effects resulting from systemic administration, decreasing dose or number of dosages and maintaining local agent levels within a desirable range [1]. One challenge to these goals is the targeting mechanisms, which can be either passive or active. Passive targeting is usually a result of the differences in the vascularization, pH and temperatures of the disease sites compared with healthy tissues, while active targeting involves the chemical decorating of the surface of drug carriers with targeting entities [2]. Another challenge is the release profiles, which are also important for therapeutic success. Preprogrammed and pulsatile release of therapeutic agents usually rely on the initial design of drug delivery system [3], while it is often preferred to alter their rate of drug delivery in response to pathological conditions such as changes in pH and temperature.

A decrease in local pH often accompanies inflammation [4], tumor growth [5] and myocardial ischemia [6] or in the gastrointestinal system [4]. Synthetic or natural polymers containing weakly acidic or basic groups have been used as the pH-sensitive controlled release systems for drug delivery [7]. And the reported pH-sensitivity is based on the neutralization of excess negative charges on their surface upon protonation [8]. Another strategy is to include pH-labile linkage in the formulation matrices, which are disrupted and release out therapeutic agents under acid environment. Guo and Szoka synthesized a pH-sensitive liposome containing ortho esters, which could also be a carrier of drugs for low pH target delivery [9]. Similarly, the anticancer drug doxorubicin was encapsulated in pH-sensitive micelles formed by incorporating hydrophobic acetal groups on the core block of a micelle-forming block copolymer. It was found that doxorubicin was selectively released at acidic pH such as those encountered in tumor tissue and in endocytic vesicles including endosomes and lysosomes [10].

Drug delivery with polymer nanofibers is based on the principle that the drug dissolution rate increases with increased surface area of both the drug and the corresponding carrier if

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necessary [11]. Electrospinning is one of the few techniques to prepare long fibers of nano to micrometer diameter. The resulting nanofibrous membrane containing drugs can be applied topically for skin and wound healing, or post-processed into other kinds of drug formulations. Both degradable and non-degradable polymers are currently under investigation to develop nanofibrous structures as drug carriers mainly for local delivery of antibiotics, antifungal, antimicrobial and anticancer drugs [12]. Bioabsorbable nanofibers of poly(lactic acid) (PLA) was used for loading an antibiotic drug Mefoxin, and the efficiency of this nanofibrous membrane compared to bulk film was demonstrated [13]. Ignatious and Baldoni defined that the release of pharmaceutical dosage from nanofibers can be designed as rapid, immediate, delayed or modified dissolution depending on the polymer carrier used [14]. We assessed the use of electrospun fibers as drug delivery vehicles with attention on the different diameters and drug encapsulated as parameters to control drug release and polymer fiber degradation [15]. For potential use in topical drug administration and wound healing, Brewster et al. investigated poorly water-soluble drugs loaded in a water-insoluble, non-biodegradable polymer [16]. The collected non-woven fabrics were shown to release the drugs at various rates and profiles based on the nanofiber morphology and drug content.

The local delivery and controllable release profiles make electrospun ultrafine fibers as potential implantable drug carriers and functional coatings of medical devices [17]. For the treatment of accessible solid tumors, fibrous formulations of anticancer drugs can be locally dosed after surgical removal. The drug eluting fibrous coatings can eliminate the intruding bacteria and alleviate the inflammatory responses after implantation of medical devices. There are few attempts to form acid-labile electrospun fibers, whose release behaviors respond to the local environment and fiber characteristics. In the current study a novel strategy was presented to synthesize biodegradable pH-sensitive polymers containing ortho ester groups. The acid-labile segments were synthesized through 3,9-dimethylene-2,4,8,10-tetraoxaspiro [5.5] undecane (DMTU) and 1,10-decanediol or PEG, which were further copolymerized with D,L-lactide (D,L-LA). The PLA segments in the polymer backbone hydrolyze to generate polymer fragments or free lactic acid containing a carboxylic acid end group, which will catalyze the ortho ester hydrolysis [18]. Biodegradable acid-labile polymers were electrospun with the encapsulation of paracetamol as a model drug, to take advantage of the pH-sensitive properties of the matrix polymer to target acid circumstances which were found in special tissue or area. *In vitro* drug release and matrix degradation were investigated in buffer solutions of pH 7.4, 5.6 and 4.0, respectively, in order to clarify the acid-responsiveness.

2. Materials and methods

2.1. Materials

Polyethylene glycol (PEG, M_w: 200 Da) was dissolved in water, extracted with dichloromethane, precipitated with ethyl ether, dried in vacuum and stored over phosphorous pentoxide prior to use. 1,10-Decanediol was dissolved in tetrahydrofuran (THF), precipitated with ethyl ether and dried in vacuum. *p*-Toluenesulfonic acid monohydrate (*p*-TSA) and D,L-LA were recrystallized from water and ethyl acetate, respectively. DMTU was prepared as described previously [19]. Stannous octoate was from Sigma–Aldrich (St. Louis, MO). Paracetamol was obtained from Kangquan Pharmaceuticals Inc., China. THF, methanol and dichloromethane were dried by refluxing and distilled before use. All other chemicals and solvents were of reagent grade or better.

2.2. Synthesis of acid-labile segments containing ortho ester groups

Ortho ester groups were introduced by reacting DMTU with 1,10-decanediol or PEG under dry nitrogen atmosphere. In a typical experiment, 1,10-decanediol (1.91 g, 11 mmol) and DMTU (1.84 g, 10 mmol) were placed into 20 ml THF. After the addition of *p*-TSA (2.4 mg, 0.014 mmol), the reaction mixture was stirred for 12 h at room temperature. The reaction was terminated by the addition of 2 ml triethylamine, and precipitated from 100 ml of methanol containing 0.5 ml of triethylamine as a stabilizer. After purified by dissolution into THF and precipitated by cold ethyl ether, the resulting polymer (POE_D) was dried in a vacuum oven, and the yield was 81%. The similar process was applied for reacting DMTU with PEG (2.2 g, 11 mmol) to obtain polymer POE_P in a 79% yield.

2.3. Synthesis of acid-labile copolymers

Copolymerization was carried out by bulk ring-opening polymerization of D,L-LA with POE_D and POE_P, respectively. Briefly, POE_D (0.1 g), D,L-LA (0.9 g) and stannous octoate (4.5 mg) were mixed, which were vacuumed and then purged with dry nitrogen for three times. After incubation at 140 °C for 8 h, the mixture was purified by repeated dissolution into dichloromethane and precipitated by cold ethyl ether. The resulting triblock polymer PLA–POE_D–PLA ($PLAOE_D$) was dried at 40 °C under vacuum, and the yield was 91%. A similar process was used for the synthesis of triblock copolymer PLA–POE_P–PLA ($PLAOE_P$) with 10% (w/w) of POE_P in a yield of 90%. As a control to acid-labile polymers, PLA was synthesized by bulk ring-opening polymerization of D,L-LA through the similar process as above.

2.4. Characterization of the obtained polymers

Fourier transform infrared spectroscopy (FTIR, Thermo Nicolet 5700, Madison, WI) and proton nuclear magnetic resonance (^{1}H NMR, Bruker Avance DPX 300, Faellanden, Switzerland) were performed to analyze polymers structure. FTIR spectra were collected over the range of 4000–400 cm^{-1} using potassium bromide (KBr) pellets or by casting polymer solutions in dichloromethane on KBr windows. The ^{1}H NMR were obtained in $CDCl_3$ with tetramethylsilane (TMS) as the internal standard. The molecular weight of POE_D, POE_P, $PLAOE_D$, $PLAOE_P$ and PLA was determined by gel permeation chromatography (GPC, waters 2695 and 2414, Milford, MA) using polystyrene with molecular weight range from 1.31 to 591 kDa as standard. Styragel HT 4 column (Waters, Milford, MA) was used and the column temperature was set as 25 °C. The mobile phase was THF using a regularity elution at a flow rate of 1.0 ml/min.

2.5. Preparation of electrospun fibers with paracetamol entrapment

The electrospinning process was performed as described elsewhere [15]. Briefly, the electrospinning was equipped with a high voltage statitron (Tianjing High Voltage Power Supply Company, Tianjing, China). The polymer and drug (98/2, w/w) were dissolved in acetone and added in a 2 ml syringe. The syringe was attached with a clinic-shaped metal capillary, which was connected to the positive pole of the high voltage statiron. The flow rate was set as 0.4 ml/h by a precision pump (Zhejiang University Medical Instrument Company, Hangzhou, China) to maintain a steady flow from the capillary outlet. The applied voltage was controlled at 20 kV, and the electrospun fibers were deposited on grounded aluminum foil fixed on a plate-type collector. The fiber collections were vacuum dried at room temperature for 2 days to completely remove any solvent residue prior to further use.

2.6. Characterization of drug loaded electrospun fibers

The morphology of the electrospun medicated fibers was examined by scanning electron microscope (SEM, Quanta 200, The Netherlands) equipped with field-emission gun (10 KV) and Robinson detector after vacuum-coated with a thin layer (10–15 nm) of gold to minimize charging effect. The fibers diameter was measured from the SEM images, and five images were used for each fibrous sample. From each image, at least 20 different fibers and 100 different segments are randomly selected and their diameter was measured to generate an average fiber diameter by using the tool of Photoshop 8.0 edition [20]. The apparent density and porosity of electrospun fibrous mats were calculated using the following equations, where the thickness of the fibrous mats was measured by a micrometer

$$\text{Apparent density } (\text{g/cm}^3) = \frac{\text{Mat mass (g)}}{\text{Mat thickness (cm)} \times \text{Mat area (cm}^2)}$$

$$\text{Mat porosity} = \left(1 - \frac{\text{Mat apparent density } (\text{g/cm}^3)}{\text{Bulk density of polymers } (\text{g/cm}^3)}\right) \times 100\%.$$

Loading of paracetamol in the ultrafine fibers was determined by extracting the drug from fiber samples. In brief, a known amount of fibers (ca. 100 mg) were dissolved in 500 μl of chloroform and extracted three times with 600 μl of double-distilled water. The drug content of the extracted solution was detected at 243 nm with an ultraviolet–visible spectrophotometer (UV-2550, Shimadzu, Japan), in which the concentration was obtained using a standard curve from known concentrations of paracetamol solutions. The extraction efficiency was calibrated by adding a certain amount of paracetamol into a polymer solution along with the same concentration as above and extracted using the above-mentioned process. The level of residual acetone within electrospun fibers was detected by gas chromatography (GC, Shanghai Analytical Instrument, China), and compared with a set of standard samples with known amount of acetone.

2.7. In vitro drug release behaviors of electrospun non-woven fabrics

The *in vitro* release profiles were investigated in buffer solutions of different pH values to clarify the acid-sensitivity [21]. The electrospun non-woven fabrics with drug entrapment were first sectioned into $20 \times 20\ \text{mm}^2$ squares and the drug content was determined as a function of scaffold weight. Triplicate fibrous samples were incubated into 20 ml of 154 mM phosphate buffered saline (pH 7.4) and acetate buffer solutions (pH 5.6, pH 4.0) containing 0.02% sodium azide as a bacteriostatic agent, respectively. The suspensions were kept in a thermostated shaking water bath (Taichang Medical Apparatus Co., Jiangsu, China) that was maintained at 37 °C and 100 cycles/min. Samples of 1.0 ml released solution were taken from the dissolution medium at predetermined intervals, while equal amount of fresh buffer solutions was added back to the incubation media. The amount of paracetamol present in release buffer was determined as described above, and the concentration was obtained using a standard curve from known concentrations of paracetamol in buffer solutions of different pH values. For standard samples with the concentrations from 0 to 20 μg/ml, linear correlations (γ^2 = 0.9999 in pH 7.4, 0.9998 in pH 5.6 and 0.9995 in pH 4.0 buffer solution) were determined between the absorption strength and paracetamol concentrations.

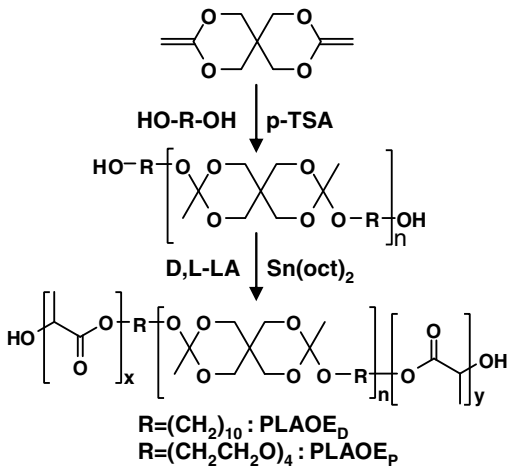
2.8. In vitro degradation behaviors of electrospun non-woven fabrics

The degradation behaviors were evaluated from the molecular weight reduction and molecular weight polydispersity (M_w/M_n) of matrix polymers, the mass loss and morphological change of fibrous mats. Pre-weighted pieces of electrospun non-woven fabrics with initial thickness of about 100 μm each and initial weight of about 100 mg were incubated at 37 °C in 20 ml of buffer solutions as described above. At predetermined intervals, triplicate samples for each kind of fabrics were recovered, washed with distilled water to remove residual buffer salts, and dried to constant weight in a vacuum oven at room temperature. The morphological change was estimated from SEM observation as mentioned above. The mass loss was determined gravimetrically by comparing the dry weight remaining at a specific time with the initial weight. The recovered and dried fabrics were dissolved in THF and filtered to eliminate insoluble residues. The molecular weight and polydispersity of the recovered matrix polymer were determined using GPC as described above.

3. Results and discussion

Generally, pH-sensitive polymers were a kind of environmentally sensitive polymers which contain carboxylic acids [22] or amines [23] and can lead to pH-dependent transition. pH-sensitive poly (β-amino ester) was developed by Langer et al. as gene carriers [24], and the pH-sensitivity came from the cationic charge inducible amine groups. These groups were protonized and deprotonized in the acid and neutral physiological conditions (pH 7.4). Another strategy was to incorporate acid disable linkages, such as acetal group [25] and hydrazone group [26], into the polymer backbone or side chains, which were stable in neutral physiological environment but wrecked in acid condition. To enhance the delivery of proteins to macrophages, Murthy et al. presented a new acid-sensitive drug delivery vehicle from poly(cyclohexane-1,4-diyl acetone dimethylene ketal), a hydrophobic polymer containing ketal linkages in the backbone [27]. Brocchini et al. developed water-soluble polyacetals derived from tyrosine-based diphenol monomers [28]. Murthy et al. designed and synthesized pH-responsive polymeric carriers, which were called "encrypted polymers" [29]. Oligonucleotides or peptides were conjugated through acetal groups onto the side chains of matrix polymer, which significantly enhanced the cytosolic delivery of fragile drugs, thus avoiding lysosomal degradation of the drugs.

Polymers with non-biodegradable backbone were used in these investigations and acid-labile groups were included as cross-linkers and linkers of the side chains. Ortho esters were another acid-labile linkage, which was suitable for applications for triggered drug release systems targeted to mildly acidic bioenvironments such as endosomes, solid tumors and inflammatory tissues. Guo and Szoka synthesized a pH-sensitive poly(ethylene glycol)-diortho ester-distearoyl glycerol conjugate, found to be stable at neutral pH for greater than 3 h but degraded completely within 1 h at pH 5 [9]. Liposomes containing the acid-labile conjugates aggregated and released most of their contents within 30 min after incubation in acidic pH as mild as 5.5. In this current study, acid-labile biodegradable polymers were designed through introducing ortho ester groups into PDLLA backbone. Scheme 1 shows the synthetic route. To examine the effect of hydrophilic acid-sensitive segments on the matrix degradation and the resulting drug release behaviors, DMTU was reacted with 1,10-decanediol and PEG, respectively, which was further copolymerized with D,L-LA to obtain acid-labile polymers with biodegradable backbone.



Scheme 1. Synthesis of acid-labile copolymers $PLAOE_D$ and $PLAOE_P$.

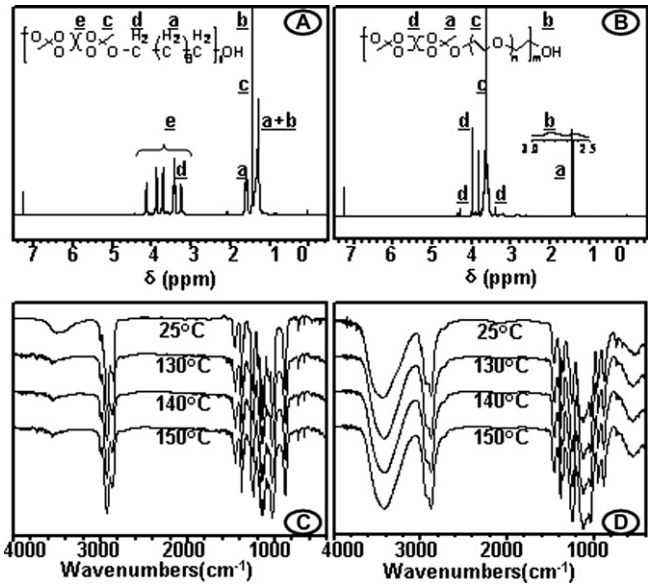


Fig. 1. ^1H NMR spectra of POE_D (A) and POE_P (B); FTIR spectra of POE_D (C) and POE_P (D) after incubation for 2 h under elevated temperatures.

3.1. Characterization of pH-sensitive segments POE_D and POE_P

Typical ^1H NMR spectra of POE_D and POE_P as well as the detailed assignment of the different peaks are shown in Fig. 1A and B, respectively. The spectrum of POE_D (Fig. 1A) showed, in addition to the hydroxyl end groups (δ = 1.3 ppm) and methylene group of 1,10-decandiol (δ = 1.3, 1.6 and 3.4 ppm) and methylene protons over the ring of DMTU (δ = 3.2–4.1 ppm), that the formation of the copolymer can be confirmed by the appearance of a sharp single peak at 1.4 ppm. The absorption peak was due to methyl protons result from the reaction between DMTU and 1,10-decandiol. The FTIR spectrum of POE_D (Fig. 1C) showed strong absorptions at 3526 cm^{-1} (O—H bond stretching), 2930 cm^{-1} and 2852 cm^{-1} (C—H stretching of 1,10-decandiol), 2879 cm^{-1} (C—H bond stretching of —CH_3), 1128 cm^{-1}, 1151 cm^{-1} and 884 cm^{-1} (C—O—C bond stretching). No peak assigned to C=C bond stretching was detected. GPC results (Fig. 2C) showed that only one peak of POE_D with weight average molecular weight (M_w) of 6500 was detected.

The spectrum of POE_P (Fig. 1B) showed, in addition to PEG (δ_{CH2} = 3.6 ppm) and protons over the ring of DMTU (δ = 3.4–4.0 ppm), that the formation of the copolymer can be confirmed by the appearance of a sharp single peak at 1.4 ppm. The FTIR spectrum of POE_P (Fig. 1D) showed strong absorptions at 3446 cm^{-1} (O—H bond stretching), 3002 cm^{-1} (C—H bond stretching of —CH_3) and 1125 cm^{-1} (C—O—C bond stretching). There was also no peak assigned to C=C bond stretching. GPC results (Fig. 4) showed only one peak of POE_P (M_w: 4000) without residual PEG.

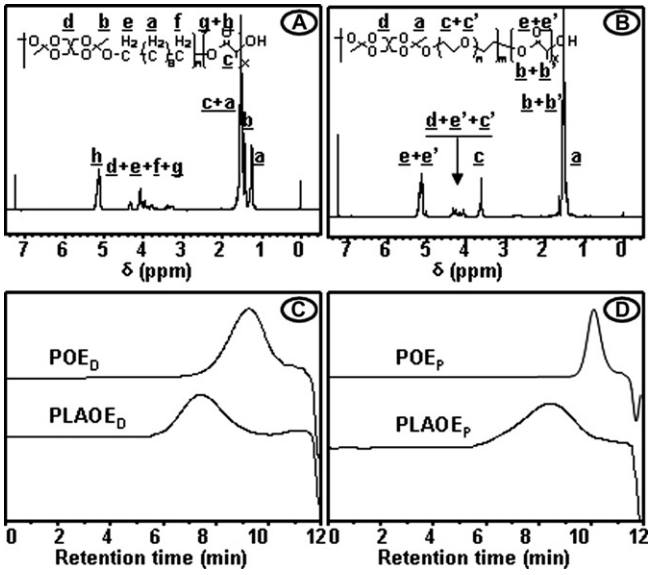


Fig. 2. ^1H NMR spectra of $PLAOE_D$ (A) and $PLAOE_P$ (B); GPC elution profiles of POE_D and $PLAOE_D$ (C), POE_P and $PLAOE_P$ (D).

It was generally thought that the temperature for bulk ring-opening polymerization of D,L-LA should be over 130 °C. In order to make sure the thermostability of POE_D and POE_P under the polymerization step, FTIR spectra were collected under elevated temperatures. KBr pellets containing acid-labile segment POE_D or POE_P were clamped by two KBr windows, which were heated from 25 to 150 °C gradually. At predetermined temperature points of 130, 140 and 150 °C, FTIR spectra were collected after baking for 2 h, respectively. Fig. 1C and D shows FTIR spectra of the acid-labile segments as a function of temperature. There was no obvious movement of wave numbers and no additional appearance or disappearance of peaks for both POE_D and POE_P.

3.2. Characterization of acid-labile polymers $PLAOE_D$ and $PLAOE_P$

Fig. 2A shows a representative ^1H NMR spectrum of $PLAOE_D$ as well as the detailed assignment of each peak. It indicated the methyl proton of LA at 1.5 ppm, methylene protons and methyl protons over ring at 3.2–4.1 and 1.4 ppm of POE_D, respectively. It should be noted that no signal at 1.3 ppm, which was assigned to the hydroxyl end groups of POE_D, was detected in the spectrum of $PLAOE_D$. The FTIR spectrum of $PLAOE_D$ showed the strong absorptions at 1755 cm^{-1} (C=O stretching), 2992 cm^{-1} (C—H stretching of —CH_3 of ester segments) and 2939 cm^{-1} (C—H stretching of —CH_2 of decane segments). NMR spectrum of $PLAOE_P$ is shown in Fig. 2B, where the methyl protons of LA at 1.5 ppm, methylene protons of PEG at 3.6 ppm, methylene protons and methyl protons over ring at 3.8–4.1 and 1.4 ppm of POE_P, respectively, are indicated. It should be noted that no signal at 2.8 ppm, which was assigned to the hydroxyl end groups of POE_P, was detected in the spectrum of $PLAOE_P$. The FTIR spectrum of $PLAOE_P$ showed the strong absorptions at 1755 cm^{-1} (C=O stretching), 2994 cm^{-1} (C—H stretching of —CH_3 of ester segments) and 2876 cm^{-1} (C—H stretching of —CH_2—CH_2—O of PEG segments).

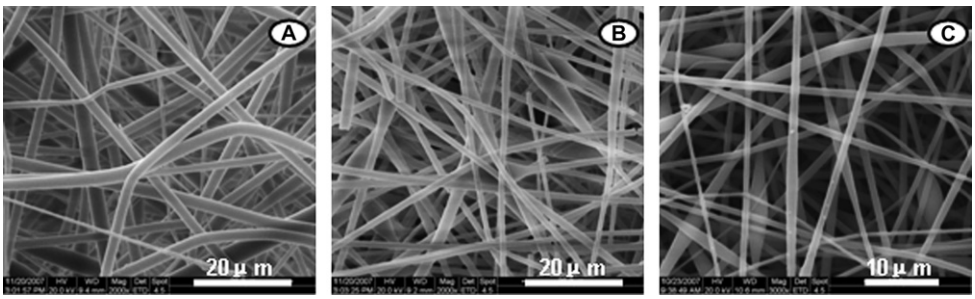


Fig. 3. SEM images of electrospun fibrous mats of PLA (A), $PLAOE_D$ (B) and $PLAOE_P$ (C) containing 2.0% of paracetamol.

The most characteristic absorption of C—O—C of PEG segments at 1120 cm^{-1} was overlapped by C—O stretching of ester segments. The formation of PLAOE copolymers was also confirmed by GPC analysis, which gave only one peak (Fig. 2C and D). Copolymers were obtained with M_w of 41 and 38 kDa and the molecular weight polydispersity (M_w/M_n) of 1.64 and 2.05 for $PLAOE_D$ and $PLAOE_P$, respectively.

3.3. Characterization of electrospun fibers

Fig. 3 shows the SEM morphologies of electrospun fibrous mats, which possessed the common feature of being round-shaped, bead-free, randomly arrayed and very porous. There no drug crystals detected on surfaces of the smooth fiber, indicating that the drug was homogeneously incorporated into the electrospun fibers. Average diameters of 940 ± 240, 1490 ± 250 and 1260 ± 250 nm were obtained for paracetamol loaded fibers of $PLAOE_D$, $PLAOE_P$ and PLA, respectively. The porosity was between 68% and 73% for all the three mats. Loaded amounts of paracetamol of 1.96 ± 0.2%, 1.94 ± 0.2% and 1.95 ± 0.3% were close to the theoretic values (2.0%, w/w) for all the fiber samples. The residual acetone was below 20 ppm evaluated by gas chromatography, which was much lower than the limit according to the USPXXIX requirement (i.e. 0.5% for acetone).

3.4. In vitro paracetamol release profiles

The drug release profile from electrospun fibers of acid-labile polymer matrices was evaluated in buffer solutions of different pH values and the results are summarized in Fig. 4. Fig. 4A shows the paracetamol release profiles from PLA fibrous mat after incubation in pH 7.4, 5.6 and 4.0 buffer solutions. Similar release behaviors were detected, and the total amounts of release were around 21%, 26% and 32% for PLA fibers during 144 h incubation in buffer solution of pH 7.4, 5.6 and 4.0, respectively. Fig. 4B and C shows the release profiles from pH-sensitive polymers $PLAOE_D$ and $PLAOE_P$ under different buffer solutions, respectively. As shown in Fig. 4B, the drug release rate from $PLAOE_D$ fibers at pH 5.6 and 4.0 became higher than that at pH 7.4, and the total amount of release was about 20%, 49% and 65% after incubation for 144 h in buffer solutions of pH 7.4, 5.6 and 4.0, respectively. The differences of release profiles were more significant between the acid and neutral buffer solutions for $PLAOE_P$ with hydrophilic segments of PEG (Fig. 4C). The total amount of release was about 65% and 72% after incubation in pH 5.6 and 4.0 buffer solutions for 144 h, respectively, while that was only 30% after incubation in pH 7.4 PBS.

As indicated in Fig. 4, the release profiles of all the fibers incubated into different pH buffer solutions were characterized by a typical biphasic pattern. A pronounced burst release was initially observed followed by a steady or gradual release during the rest time. The burst release was contributed by the drug of paracetamol dispersing close to the surface of polymer fibers and adsorbing or loosely binding near the surface, which diffused out quickly in initial incubation time. There was about 15–24% of the loading amount that initially released out for PLA, $PLAOE_D$ and $PLAOE_P$ fibers after incubation in pH 7.4 PBS. But in acid circumstance, the amounts of burst release of $PLAOE_D$ and $PLAOE_P$ fibers were increasingly higher. After incubation in pH 5.6 buffer solution around 17%, 31% and 36% of the paracetamol release were detected in the initial 12 h for PLA, $PLAOE_D$ and $PLAOE_P$ fibers, respectively. This may be due to promoting the erosion of fiber surface and the detachment of drug from fiber matrix under acid condition.

The sustained release from the polymer matrix was mainly controlled by not only the drug diffusion from the matrix but also the degradation of the matrix [30]. The porous structure of fibrous mats and the micropores after the diffusion out of drug from the polymers made it possible that further constant release of drug from inner matrix. As shown in Fig. 4, there was no significant difference in the sustained release of paracetamol among PLA, $PLAOE_D$ and $PLAOE_P$ fibers after incubation in pH 7.4 PBS. About 6% of the loading amount was released from PLA, $PLAOE_D$ and $PLAOE_P$ fibers during incubation time from 12 to 144 h in pH 7.4 PBS, respectively (Fig. 4). Similar results were also observed for PLA fibers after incubation in buffer solutions of different pH values

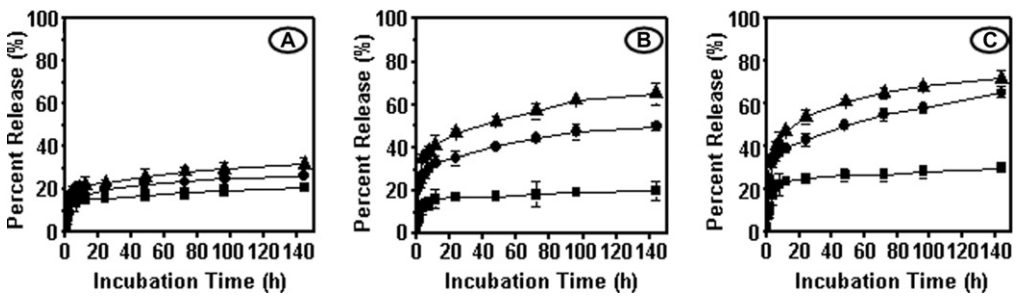


Fig. 4. Release profiles of paracetamol from PLA (A), $PLAOE_D$ (B) and $PLAOE_P$ (C) fibrous mats with 2.0% of paracetamol entrapment in pH 7.4 (■), pH 5.6 (●) and pH 4.0 (▲) buffer solutions at 37 °C ($n = 3$).

(Fig. 4A). All the polymer matrices were relatively stable under these incubation conditions, and amount of drug still entrapped into the fibers, which would not release out until significant degradation of matrix polymer occurred [31]. However, when the pH value of buffer solutions was 5.6 and 4.0, the amount of drug released from pH-sensitive polymer fibers was accelerated during the sustained release phase. About additional 9%, 18% and 29% of the loading amount were released from PLA, $PLAOE_D$ and $PLAOE_P$ fibers, respectively, during incubation time from 12 to 144 h in pH 5.6 buffer solution. The pores left after initial burst release and subsequent drug diffusion were critical for further release from the inner sections of fibers through the swollen and porous inner structure. This could be enhanced through the matrix breakdown of the acid-labile polymers in acid environment. As shown in Fig. 4B and C, $PLAOE_P$ fibers exhibited higher sustained release rate than $PLAOE_D$ fibers under acid buffer solutions. This was due to the relative hydrophilic surface, which facilitated water diffusion in and drug diffusion out of the fiber matrix.

Thus, it may be concluded that larger initial burst release and higher sustained release rate can be achieved in lower pH environment, which was actually ideal since it was important to inhibit the tumor cell growth for cancer treatment and to eliminate the intruding bacteria before they began to proliferate for inflammation control. Delayed release behaviors obtained from the above investigation demonstrated a potential for programmed delivery by combination of fibers with different acid-labile segments for clinical needs. The release rate and the length of sustained release can be adjusted to closely relate with the local pathological conditions.

3.5. In vitro degradation behaviors electrospun fibrous mats

The processes involved in the erosion of a degradable polymeric fiber are complicated. The high water repellent property of electrospun fiber led to much slower water penetration into the fibrous mat [20]. Water enters the fibers bulk, which might be accompanied by swelling. The intrusion of water triggers the chemical polymer degradation, leading to the creation of oligomers and monomers. Progressive degradation changes the microstructure of the fiber matrix through the formation of micropores, via which oligomers and monomers are released. So the inclusion of acid-labile segments with different hydrophilicity into PLA backbone is the most important factors that influence the water uptake, degradation velocity and acid-sensitivity.

Degradation profiles of the fibrous mats were assessed macroscopically by SEM. In contrast to the smooth surface of ultrafine fibers before incubation as shown in Fig. 3, significant changes of the morphologies of fibrous mats were detected. Fig. 5 shows the morphologies of electrospun PLA, $PLAOE_D$ and $PLAOE_P$ fibrous mats at 24 h after incubation in different media. Electrospun PLA fibers were swollen and curly, and fibers space shrunk for all samples (Fig. 5A, B and C) compared with original formation (Fig. 3A). This was due to the chain relaxation of matrix polymer after incubation into the medium with elevated temperature [15]. And the shrinkage and congeries of fibers could be easily found for $PLAOE_D$ and $PLAOE_P$ fibrous mats after incubation for 24 h in buffer solutions of pH 7.4 (Fig. 5D and G). Morphological coexistence of fibers and films could be found for electrospun $PLAOE_D$ mats after incubation in pH 5.6 buffer solution for 24 h (Fig. 5E), and for $PLAOE_P$

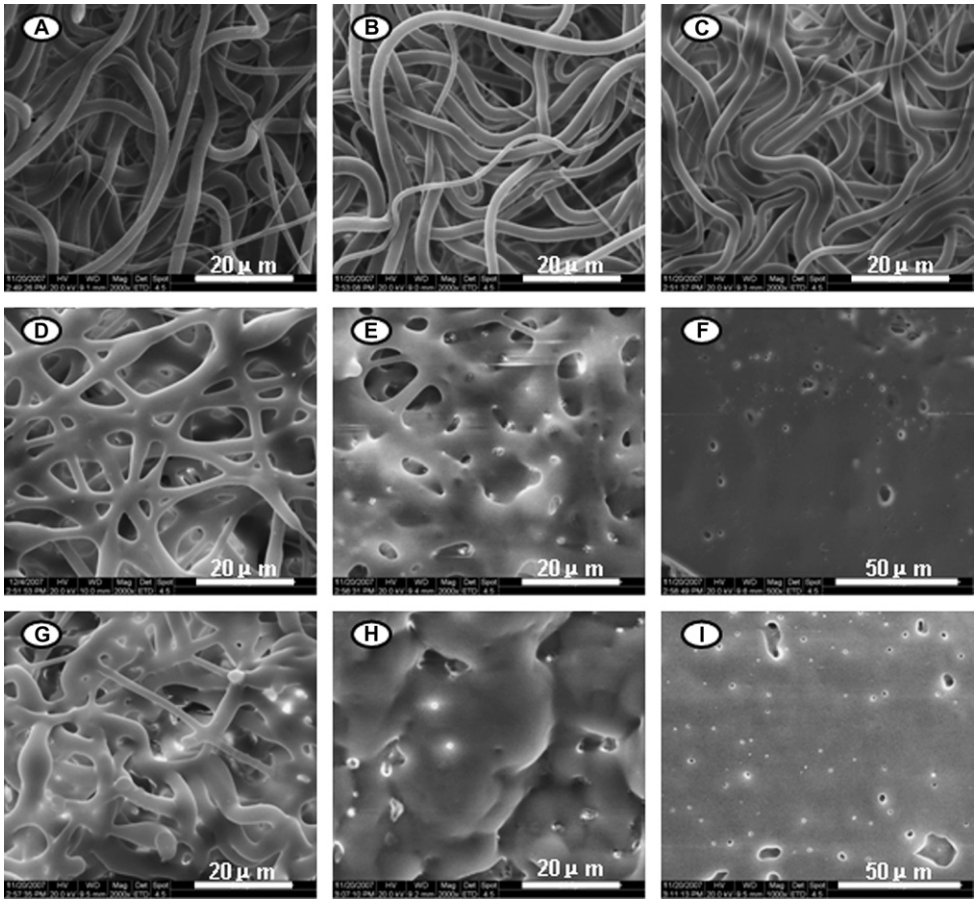


Fig. 5. SEM images of paracetamol loaded fibrous mats of PLA after incubation for 24 h at 37 °C in pH 7.4 (A), 5.6 (B) and 4.0 (C), of $PLAOE_D$ mats in pH 7.4 (D), 5.6 (E) and 4.0 (F) and of $PLAOE_P$ mats in pH 7.4 (G), 5.6 (H) and 4.0 (I) buffer solutions.

mats in pH 7.4 (Fig. 5G). Higher sensitivity of fibers morphology to lower pH environment was observed for $PLAOE_P$ fibrous mats, and polymeric films without evident fiber morphologies were detected for $PLAOE_D$ and $PLAOE_P$ mats incubated in pH 4.0 buffer solutions (Fig. 5F and I).

The degradation behaviors of $PLAOE_D$, $PLAOE_P$ and PLA fibers were determined in buffer solutions of different pH values with regard to the mass loss of fiber matrices, the molecular weight reduction and molecular weight polydispersity of the matrix polymers. Gravimetric evaluation of the mass loss during incubation is summarized in Fig. 6a. There were about 7% of mass loss for electrospun PLA fibrous mats after incubation for 72 h in buffer solutions of pH 7.4, 5.6 and 4.0, respectively (Fig. 6a1). The mass loss of the fibrous mats may be resulted from the dissolution of oligomers into the medium, and more oligomers or scraps could be formed due to faster degradation of matrix polymer after incubation into low pH solutions. Around 13% of mass loss for $PLAOE_D$ (Fig. 6a2) and 19% for $PLAOE_P$ (Fig. 6a3) were detected in pH 4.0 buffer solutions, respectively. Fig. 6b showed the molecular weight reduction of matrix polymers of fibrous residuals. Less than 10% of molecular weight reduction was found for PLA fibrous mat in three kinds of buffer solutions, and similar results could be found for $PLAOE_D$ and $PLAOE_P$ mats in pH 7.4 buffer solution. The molecular weight decreased gradually with incubation time and no significant difference was found for $PLAOE_D$ and $PLAOE_P$ mats after incubation into pH 7.4 buffer solution. There were around 17% and 23% of molecular weight loss for $PLAOE_D$ mats, and 24% and 31% for $PLAOE_P$ after incubation for 72 h in pH 5.6 and 4.0 buffer solutions, respectively. As indicated above, the degradation behaviors of $PLAOE_D$ and $PLAOE_P$ fibrous mats in pH 7.4 buffer solution were close to PLA mats, while significantly higher mass loss and molecular weight reduction were detected after incubation into acidic buffer solutions. It indicated that electrospun fibers of PLAOE containing acid-labile segments were as "stable" as PLA without acid-labile segments under neutral buffer solution, but were highly sensitive to the acid environment. An increase in polydispersity was observed for all samples (Fig. 6c) paracetamol loaded PLA electrospun fibers showed approximately 8% increase after incubation for 72 h in pH 7.4, 5.6 and 4.0 buffer solutions, respectively. Significantly larger molecular weight distribution was detected for matrix polymer with inclusion of acid-labile segments. And there were about 2%, 9% and 14% increase for $PLAOE_D$, about 12%, 16% and 21% of increase for $PLAOE_P$ after incubation in pH 7.4, 5.6 and 4.0 buffer solutions, respectively. The acid-labile segment of PLAOE contributed to the degradation during the investigational period, and the breakdown of the ABA copolymer backbone of the matrix polymer resulted in significant increase in the molecular weight polydispersity.

The degradation behavior of matrix polymer is one of the most important aspects for the end application as drug carriers. The differences between the breakdown velocity of chemical bond of matrix polymer and the distribution speed of water into the polymer matrix would determine the degradation mechanism for most of the degradable polymers. The degradation of electrospun fibrous

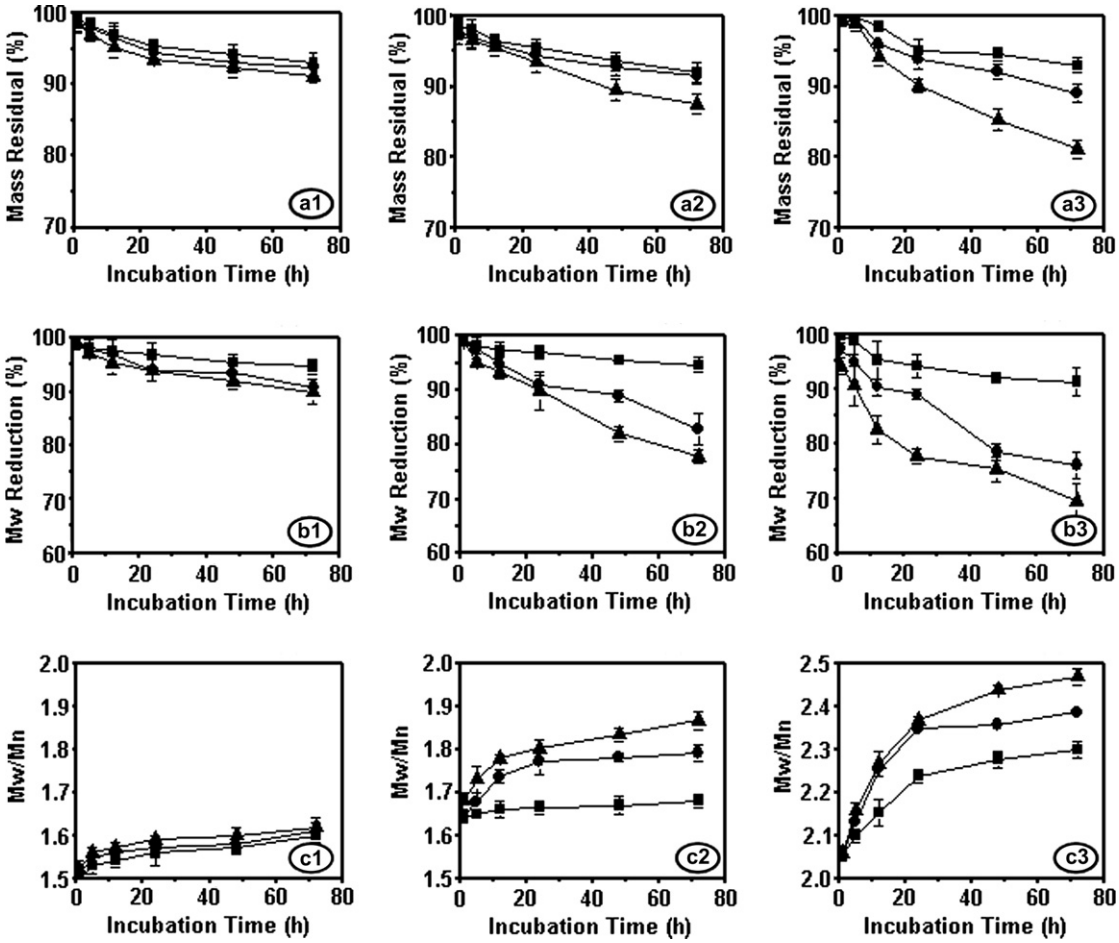


Fig. 6. The residual mass percent of fibrous mats (a), molecular weight reduction (b) and molecular weight polydispersity (M_w/M_n) (c) of matrix residuals of PLA (a1, b1 and c1), $PLAOE_D$ (a2, b2 and c2) and $PLAOE_P$ (a3, b3 and c3) fibers with 2.0% of paracetamol entrapment after incubation in pH 7.4 (■), 5.6 (●) and 4.0 (▲) buffer solutions at 37 °C ($n = 3$).

mats showed different patterns from solvent casting films and other bulk formulations due to the electrospinning process [32]. As shown in Fig. 6, the mass loss and molecular weight reduction for electrospun polymer fibers were comparative, which showed a degradation pattern between surface erosion and typical bulk degradation [32]. The breakdown of the polymer backbone and the mass loss of the fibrous mat would enhance the diffusion of drug out of the fiber matrix. As shown in Fig. 6, the mass loss and molecular weight reduction of $PLAOE_D$ and $PLAOE_P$ after incubation in pH 7.4 buffer solutions were not significant, and similar profiles were also observed for PLA fibers after incubation in three buffers. This resulted in the limited amount of sustained release during the investigation period. The degradation was enhanced for PLAOE under acid conditions, and significantly higher amount of sustained release was detected. On the other hand, the matrix degradation and water swelling led to the morphological changes of the fibrous mats to collapsed films (Fig. 5), which resulted in significant decrease in effective surface area for drug release. This may cause a delayed release, and the sustained release rates of paracetamol were not as high as expected from acid-labile matrix under acid conditions. The integrative effect of the degradation process, along with the drug concentration gradient, was supposed to result in the sustained release profiles as shown in Fig. 4.

4. Conclusions

A novel strategy was developed to synthesize acid-labile polymers by incorporating ortho ester groups into biodegradable backbone. *In vitro* release study showed that the amount of initial burst release and the sustained release rate from electrospun medicated fibers were controlled through inclusions of acid-labile segments with different hydrophilicity and the local acid environment. *In vitro* degradation study indicated that the electrospun fibers containing acid-labile segments were stable in neutral buffer solutions, and the degradation was accelerated under acid circumstances. The matrix degradation led to accelerated release due to the enhanced diffusion but delayed release due to decrease in effective surface area, and the integrative effect was supposed to result in the sustained release profiles.

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References

[1] M.J. Vicent, R. Duncan, Polymer conjugates: nanosized medicines for treating cancer, Trends Biotechnol. 24 (2006) 39–47.
[2] V.A. Sethuraman, Y.H. Bae, TAT peptide-based micelle system for potential active targeting of anti-cancer agents to acidic solid tumors, J. Control. Release 118 (2007) 216–224.
[3] S. Sershen, J. West, Implantable, polymeric systems for modulated drug delivery, Adv. Drug Deliver. Rev. 54 (2002) 1225–1235.
[4] M. Kollarik, F. Ru, B.J. Undem, Acid-sensitive vagal sensory pathways and cough, Pulm. Pharmacol. Ther. 20 (2007) 402–411.
[5] L.E. Gerweck, Tumor pH: implications for treatment and novel drug design, Semin. Radiat. Oncol. 8 (1998) 176–182.
[6] J. Levitsky, D. Gurell, W.H. Frishman, Sodium ion/hydrogen ion exchange inhibition: a new pharmacologic approach to myocardial ischemia and reperfusion injury, J. Clin. Pharmacol. 38 (1998) 887–897.
[7] C.L. Lo, C.K. Huang, K.M. Lin, G.H. Hsiue, Mixed micelles formed from graft and diblock copolymers for application in intracellular drug delivery, Biomaterials 28 (2007) 1225–1235.
[8] H. Karanth, R.S.R. Murthy, pH-sensitive liposomes principle and application in cancer therapy, J. Pharm. Pharmacol. 59 (2007) 469–483.
[9] X. Guo, F.C. Szoka Jr., Steric stabilization of fusogenic liposomes by a low-pH sensitive PEG-diortho ester–lipid conjugate, Bioconjug. Chem. 12 (2001) 291–300.
[10] E.R. Gillies, J.M. Frechet, pH-responsive copolymer assemblies for controlled release of doxorubicin, Bioconjug. Chem. 16 (2005) 361–368.
[11] F. Yang, R. Murugan, S. Wang, S. Ramakrishna, Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering, Biomaterials 26 (2005) 2603–2610.
[12] K. Kim, Y.K. Luu, C. Chang, D. Fang, B.S. Hsiao, B. Chu, M. Hadjiargyrou, Incorporation and controlled release of a hydrophilic antibiotic using poly(lactide-co-glycolide)-based electrospun nanofibrous scaffolds, J. Control. Release 98 (2004) 47–56.
[13] Y. Zhang, C.T. Lim, S. Ramakrishna, Z. Huang, Recent development of polymer nanofibers for biomedical and biotechnological applications, J. Mater. Sci. Mater. Med. 16 (2005) 933–946.
[14] F. Ignatious, J.M. Baldoni, Electrospun pharmaceutical compositions, EP1251829, 2002.
[15] W. Cui, X. Li, X. Zhu, G. Yu, S. Zhou, J. Weng, Investigation of drug release and matrix degradation of electrospun poly(DL-lactide) fibers with paracetamol inoculation, Biomacromolecules 7 (2006) 1623–1629.
[16] G. Verreck, I. Chun, J. Rosenblatt, J. Peeters, A.V. Dijck, J. Mensch, M. Noppe, M.E. Brewster, Incorporation of drugs in an amorphous state into electrospun nanofibers composed of a water-insoluble, nonbiodegradable polymer, J. Control. Release 92 (2003) 349–360.
[17] A. Greiner, J.H. Wendorff, Electrospinning: a fascinating method for the preparation of ultrathin fibers, Angew. Chem. Int. Ed. 46 (2007) 5670–5703.
[18] K. Schwach-Abdellaoui, J. Heller, R. Gurny, Hydrolysis and erosion studies of autocatalyzed poly(ortho esters) containing lactoyl-lactyl acid dimers, Macromolecules 32 (1999) 301–307.
[19] J. Yu, Z. Lu, H. Zheng, R. Zhuo, Synthesis and characterization of new ointment-like poly(ortho esters), Eur. Polym. J. 38 (2002) 971–975.
[20] W. Cui, X. Li, S. Zhou, J. Weng, Investigation on process parameters of electrospinning system through orthogonal experimental design, J. Appl. Polym. Sci. 103 (2007) 3105–3112.
[21] Y. Chan, V. Bulmus, M.H. Zareie, F.L. Byrne, L. Barner, M. Kavallaris, Acid-cleavable polymeric core-shell particles for delivery of hydrophobic drugs, J. Control. Release 115 (2006) 197–207.
[22] M.A. Yessine, C. Meier, H.U. Petereit, J.C. Leroux, On the role of methacrylic acid copolymers in the intracellular delivery of antisense oligonucleotides, Eur. J. Pharm. Biopharm. 63 (2006) 1–10.
[23] T.G. Park, J.H. Jeong, S.W. Kim, Current status of polymeric gene delivery systems, Adv. Drug Deliver. Rev. 58 (2006) 467–486.
[24] S.R. Little, D.M. Lynn, S.V. Puram, R. Langer, Formulation and characterization of poly (β amino ester) microparticles for genetic vaccine delivery, J. Control. Release 107 (2005) 449–462.
[25] N. Murthy, J. Campbell, N. Fausto, A.S. Hoffman, P.S. Stayton, Design and synthesis of pH-responsive polymeric carriers that target uptake and enhance the intracellular delivery of oligonucleotides, J. Control. Release 89 (2003) 365–374.
[26] H.S. Yoo, E.A. Lee, T.G. Park, Doxorubicin-conjugated biodegradable polymeric micelles having acid-cleavable linkages, J. Control. Release 82 (2002) 17–27.
[27] S. Lee, S.C. Yang, M.J. Heffernan, W.R. Taylor, N. Murthy, Polyketal microparticles: a new delivery vehicle for superoxide dismutase, Bioconjug. Chem. 18 (2007) 4–7.
[28] J. Rickerby, R. Prabhakar, M. Ali, J. Knowles, S. Brocchini, Water-soluble polyacetals derived from diphenols, J. Mater. Chem. 15 (2005) 1849–1856.
[29] N. Murthy, J. Campbell, N. Fausto, A.S. Hoffman, P.S. Stayton, Bioinspired pH-responsive polymers for the intracellular delivery of biomolecular drugs, Bioconjug. Chem. 14 (2003) 412–419.
[30] H. Seong, T.K. An, G. Khang, S.U. Choi, C.O. Lee, H.B. Lee, BCNU-loaded poly(D,L-lactide-co-glycolide) wafer and antitumor activity against XF-498 human CNS tumor cells in vitro, Int. J. Pharm. 251 (2003) 1–12.
[31] X. Li, X. Deng, Z. Huang, In vitro protein release and degradation of poly-DL-lactide-poly (ethylene glycol) microspheres with entrapped human serum albumin: quantitative evaluation of the factors involved in protein release phases, Pharm. Res. 18 (2001) 117–124.
[32] W.G. Cui, X.H. Li, S.B. Zhou, J. Weng, Degradation patterns and surface wettability of electrospun fibrous mats, Polym. Degrad. Stabil. 93 (2008) 731–738.