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Electrospun fibers of acid-labile biodegradable polymers with acetal groups as potential drug carriers

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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. Till date there is no literature report on drug delivery from acid-labile electrospun fibers, whose degradation and drug release behaviors respond to the local pathological pH environment. Acid-labile groups have been incorporated into nonbiodegradable backbones as crosslinkers or linkers of the side chains. A novel strategy was developed in this study to synthesize acid-labile polymers by introducing acetal groups into biodegradable backbone of poly(DL-lactide)–poly(ethylene glycol). *In vitro* release study showed that the total amount of drug released from acid-labile polymeric fibers was accelerated on account of pH-induced structural and morphological changes of fibrous mats and the degradation of matrix polymers, and the burst release was significant higher for polymers with higher contents of acid-labile segments. During the investigational period, almost no molecular weight reduction and mass loss was detected in neutral buffer solutions, but the degradation was enhanced in acid buffers with a two-stage degradation profile. Surface erosion mechanism was initially detected for fibrous mats with distinct fiber morphologies, and bulk degradation was determined during the following incubation for polymeric films resulting from the morphological changes.

1. Introduction

In recent years, research and development in the field of drug delivery systems facilitating site-specific therapy has achieved significant progress. Drug delivery and targeting systems aim to minimize drug degradation and loss, to prevent harmful side effects and increase the availability of the drug at the disease site (Vicent and Duncan, 2006). Drug carriers include microparticles and nanoparticles, microcapsules and nanocapsules, liposomes, and micelles (Nakayama et al., 2006). The main advantage of fibrous carriers is that it offers site-specific delivery of any number of drugs from the scaffold into the body. With respect to the local delivery of bioactive ingredient, such as growth factors and genes, electrospun ultrafine fibers have been investigated as tissue engineering scaffold and as biofunctional scaffolds for other biomedical applications (Yoshimoto et al., 2003; Luu et al., 2003). Li et al. (2006) studied the electrospun silk fibroin fibers containing bone morphogenetic protein 2 (BMP-2) as bone tissue engineering scaffold, and higher calcium deposition and upregulation of BMP-2 transcript levels were detected compared with the control group. Both degradable and nondegradable polymers are currently under investigation to develop nanofibrous structures as drug carriers mainly for local delivery of antibiotics, antifungal, antimicrobial, and anticancer drugs (Xie and Wang, 2006). Zeng et al. examined the release profiles of different model drugs, paclitaxel, doxorubicin hydrochloride and doxorubicin base, from poly(L-lactide) electrospun fibers. The burst release of the drugs can be avoided by using compatible drugs with polymers, and the drug release can follow nearly zero-order kinetics (Zeng et al., 2005). Kenawy et al. (2002) reported electrospun nanofibers of poly(ethylene-*co*-vinylacetate), poly(lactic acid), and a blend to encapsulate tetracycline hydrochloride for the treatment of periodontal disease. We assessed the use of electrospun fibers as drug delivery vehicles with attention on the different diameters and drug contents as parameters to control drug release and polymer fiber degradation (Cui et al., 2006).

Drug release profiles, which are also important for therapeutic success, are often preferred responding to pathological conditions, such as changes in pH or temperature. The decrease of

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pH is implicated in many physiological and pathological progressions such as inflammation (Gallin et al., 1992), tumor growth (Gerweck, 1998), and myocardial ischemia (Levitsky et al., 1998). A variety of synthetic or natural polymers containing the weakly acidic or basic groups have been employed as the pH-sensitive controlled release systems for drug delivery (Na et al., 2006). Generally, the reported pH-sensitive liposomes are based on the neutralization of excess negative charges on their surface upon protonation (Karanth and Murthy, 2007). pH-sensitive liposomes containing *ortho* esters could also be a carrier of drugs for low pH target delivery (Guo and Szoka, 2001). Alternatively, the anticancer drug doxorubicin was encapsulated in pH-sensitive micelles formed from a poly(ethylene oxide)-dendritic polyester copolymer with acid-labile acetal groups on the core-forming dendrimer periphery. It was found that the acetal groups undergo hydrolysis and that doxorubicin was selectively released at acidic pH such as those encountered in tumor tissue and in endocytic vesicles including endosomes and lysosomes (Gillies and Frechet, 2005).

The local delivery and controllable release profiles make electrospun ultrafine fibers as potential implantable drug carriers and functional coatings of medical devices (Greiner and Wendorff, 2007). Till date there is no literature report on drug delivery from acid-labile electrospun fibers, whose release behaviors respond to the local environment and fiber characteristics. In this study a novel strategy was developed to synthesize biodegradable pH-sensitive polymers through introducing acetal groups into the backbone of poly(DL-lactide)–poly(ethylene glycol) (PELA). Currently pH-sensitive polymers were constructed by incorporating such groups as amines (Park et al., 2006) or carboxylic acids (Kyriakides et al., 2002) into the side chains or backbone, which were protonized or deprotonized in the physiological relevant pH range of 5.0–7.4. Langer et al. developed pH-sensitive poly(β-amino ester) as gene carriers (Little et al., 2005), and the pH-sensitivity came from the cationic charge inducible amine groups. Another strategy was to incorporate acid degradable linkages, such as acetal (Murthy et al., 2003a) and hydrazone (Bae et al., 2005), into the polymers, which were stable in physiological environment but broken down in acid conditions. Brocchini et al. developed water-soluble polyacetals derived from tyrosine-based diphenol monomers (Rickerby et al., 2005). Murthy et al. (2003b) designed and synthesized pH-responsive polymeric carriers, which called "encrypted polymers". Biomacromolecules were conjugated through acetal groups onto the side chains of matrix polymer, which significantly enhanced the delivery of oligonucleotides and peptides to the cytoplasm of cultured macrophages. 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 (Lee et al., 2007). Polymers with nonbiodegradable backbone were used in these investigations and acid-labile groups were included as crosslinkers and linkers of the side chains.

In the present study, acetal groups were introduced by reacting PEG with benzaldehyde to obtain the prepolymer PBE, which was further copolymerized with LA to obtain PBELA with biodegradable backbone. The content of acetal groups of PBELA was adjusted with the inclusion amount of PBE into the copolymerization process. And ultrafine fibers were electrospun with the encapsulation of paracetamol as a model drug, to take advantage of the pH-sensitive properties of the matrix polymer to regulate the release. *In vitro* drug release and matrix degradation were investigated in buffer solutions of pH 7.4, 5.5 and 4.0, respectively, to clarify the acid-sensitivity.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol) 200 (PEG200, M_w = 200 Da) and PEG2000 (M_w = 2000 Da) were dissolved in water, extracted with methylene dichloride, precipitated with ethyl ether, dried in vacuum and stored over phosphorus pentoxide prior to use. D,L-Lactide (D,L-LA) were recrystallized from toluene, and *p*-toluenesulfonic acid monohydrate (*p*-TSA) from water just before use. Benzaldehyde, washed by 5% sodium carbonate, and tetrahydrofuran (THF), dried by refluxing with sodium, were distilled under dry nitrogen atmosphere before use. Molecular sieve (5 Å) was activated by incubation in oven at 500 °C for 4 h. Paracetamol was obtained from Kangquan Pharmaceuticals Inc., China. All other chemicals and solvents were of reagent grade or better.

2.2. Synthesis of acid-labile polymers

Acetal groups were introduced by reacting PEG200 with benzaldehyde under dry nitrogen atmosphere. In a typical experiment, benzaldehyde (1.06 g, 10 mmol), PEG200 (2.20 g, 11 mmol), and molecular sieves (25 g) were placed into 50 ml THF. After the addition of *p*-TSA (0.85 g, 5 mmol), the reaction mixture was stirred for 12 h in an ice bath. The reaction was stopped by the addition of triethylamine (15 ml, 108 mmol). The resulting polymer (PBE) was precipitated from 200 ml of ethyl ether containing 0.5 ml of triethylamine as the stabilizer, and dried in a vacuum oven.

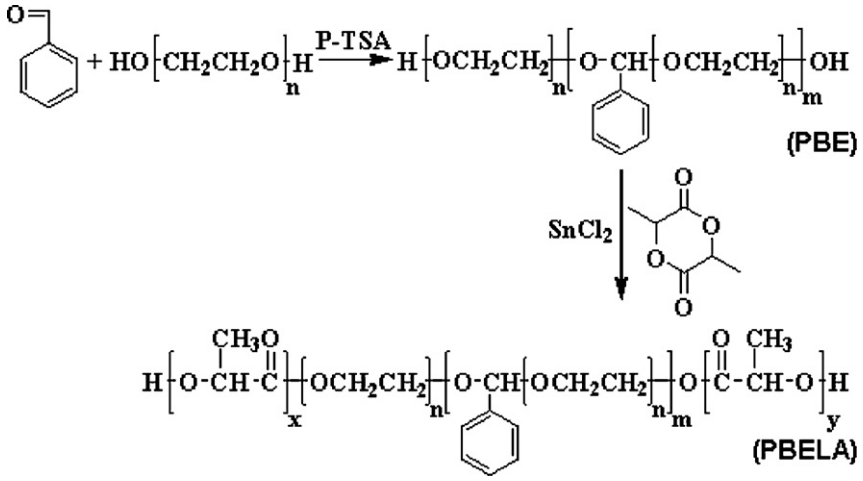
Copolymerization of PBE and D,L-LA were carried out by bulk ring-opening polymerization of D,L-LA with PBE contents of 5 and 10% (w/w), respectively. Briefly, PBE (0.1 g), D,L-LA (0.9 g) and stannous chloride (4.5 mg) were mixed, which were vacuumed and then purged with dry nitrogen for three times. After incubated at 140 °C for 8 h, the mixture were purified by repeated dissolution into dichloromethane and precipitated by cold ethyl ether. The resulting triblock copolymer PLA–PBE–PLA (PBELA) was dried at 40 °C under vacuum, and the yield was 93%. A similar process was used for the synthesis of triblock copolymer PLA–PEG–PLA (PELA) with 10% (w/w) of PEG2000 as described above.

2.3. Polymers characterization

The molecular weights of PBE, PELA and PBELA were determined by gel permeation chromatography (GPC, Waters 2695 and 2414, Milford, MA). PELA and PBELA were detected with Styragel HT 4 column (Waters, Milford, MA) using polystyrene as standard. The mobile phase was THF using a regularity elution at a flow rate of 1.0 ml/min. Ultrahydrogel 250 column (Waters, Milford, MA) was applied for PBE detection using poly(ethylene glycol) as standard, and the mobile phase was methanol and water (1/9, v/v) at a flow rate of 0.5 ml/min. Proton nuclear magnetic resonance (^{1}H NMR, Bruker Avance DPX 300, Faellanden, Switzerland) and Fourier transform infrared spectroscopy (FTIR, Thermo Nicolet 5700, Madison, WI) were performed to analyze the polymer structure. The FTIR spectra were collected over the range of 4000–400 cm^{-1} using potassium bromide (KBr) pellets or by casting polymer solutions in CH_2Cl_2 on KBr windows. The ^{1}H NMR spectra were obtained at room temperature in $CDCl_3$ with tetramethylsilane as an internal standard.

2.4. Preparation of electrospun fibers with paracetamol entrapment

The electrospinning process was performed as described elsewhere (Cui et al., 2007). Briefly, the electrospinning was



Scheme 1. Synthesis of PBE and PBELA.

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 THF and added in a 2-ml syringe, attached with a clinic-shaped metal capillary. The flow rate was controlled within 0.3–0.8 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 within the range of 10–30 kV. The fiber collections were vacuum dried at room temperature for 2 days to completely remove any solvent residue prior to further use.

2.5. Characterization of paracetamol loaded electrospun fibers

The morphology of the electrospun medicated fibrous was investigated by scanning electron microscope (SEM, FEI Quanta 200, the Netherlands) equipped with field-emission gun (10 kV) and Robinson detector after 2 min of gold coating 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 were randomly selected and their diameter measured to generate an average fiber diameter by using the tool of Photoshop 8.0 edition (Cui et al., 2007). 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 UV–vis 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 crystalline states of paracetamol, polymer fibers and paracetamol/polymer fibers were analyzed by X-ray diffraction (XRD, Philips X'Pert PRO, the Netherlands). The samples were scanned from 5° to 60° at a scanning rate of 5°/min, using Cu Kα radiation (λ = 1.54060 Å). The differential scanning calorimeter (DSC, Netzsch STA 449C, Bavaria, Germany) measurements were performed in perforated and covered aluminum pans under a nitrogen purge. Approximately 1.5 mg of polymer fiber sample was heated from 25 to 150 °C with a heating rate of 10 °C/min.

2.6. 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 (Chan et al., 2006). The electrospun non-woven fabrics with drug entrapment were first sectioned into 20 mm × 20 mm squares and the drug content was determined as a function of scaffold weight. Triplicate fibrous samples were incubated into 20.0 ml of 154 mM phosphate buffered saline (pH 7.4) and acetate buffer solutions (pH 5.5, 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 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h after incubation, respectively, 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 as 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 5.5, and 0.9995 in pH 4.0 buffer solution) were determined between the absorption strength and paracetamol concentration. And the molar absorption coefficients (ε) were 2.098×10^5, 2.254×10^5 and 2.337×10^5 l/mol cm for paracetamol in pH 7.4, 5.5 and 4.0 buffer solutions, respectively. The structural integrity of released paracetamol was determined by high-performance liquid chromatography (HPLC) with ultraviolet detector set at 243 nm (Waters 2695 and 2487, Milford, MA) using fresh paracetamol as control. The mobile phase consisted of a phosphate buffer (pH 4.5) and methanol (80/20, v/v) at a flow rate of 1.0 ml/min.

2.7. In vitro degradation behaviors

The degradation behaviors were evaluated from the morphological changes, the mass loss, the molecular weight reduction and the polydispersity (M_w/M_n). Pre-weighted pieces of electrospun non-woven fabrics with initial thickness of about 150 μm each and initial weight of about 80 mg were incubated at 37 °C in 20.0 ml of buffer solutions as described above. At predetermined intervals, triplicate samples were recovered, rinsed with distilled water to remove residual buffer salts, and dried to constant weight in a vac-

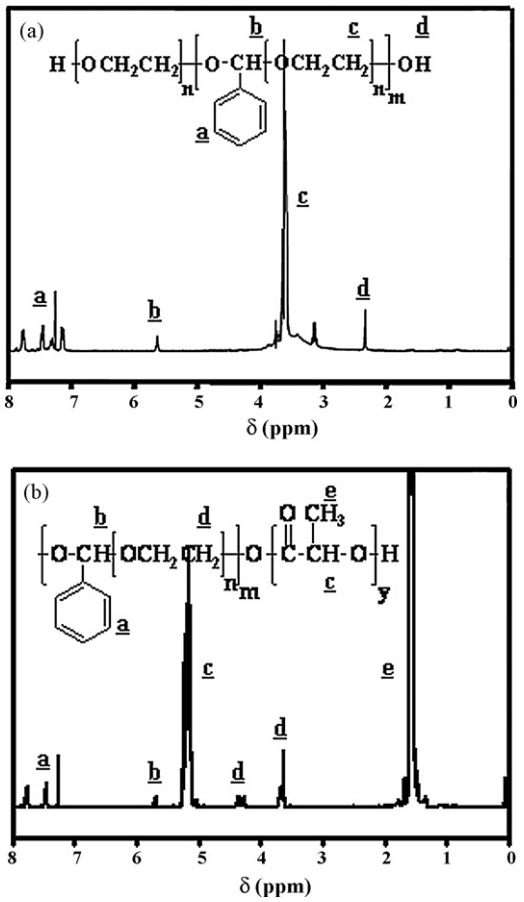


Fig. 1. ^{1}H NMR spectra of PBE (a) and PBLEA (b).

uum desiccator. The morphological changes were 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 recovered matrix polymer were determined using GPC, and the structural integrity of the polymer residues was analyzed by ^{1}H NMR and FTIR as mentioned above.

3. Results and discussion

3.1. Characterization of copolymers PBELA

Scheme 1 shows the synthetic route of PBELA. A typical ^{1}H NMR spectrum of PBE as well as the detailed assignment of the different peaks is shown in Fig. 1a. The spectrum showed, in addition to PEG (δ_{CH_2} = 3.6 ppm) and phenyl of benzaldehyde (δ = 7.2 ppm), the formation of the copolymer can be confirmed by the appearance of a sharp single peak at 5.6 ppm. The absorption peak was due to methine protons of acetal group of PBE, which was different from that of aldehyde group of benzaldehyde (δ = 10.0 ppm). The FTIR spectrum of PBE showed strong absorptions at 3382 cm^{-1} (O–H stretching), 3060 and 1645 cm^{-1} (=C–H and C=C stretching of phenyl), 812, 688 and 572 cm^{-1} (=C–H bending of phenyl), and 1130 cm^{-1} (C–O–C stretching of PEG segments). GPC results (Fig. 2a) showed that only one peak of PBE with weight average molecular weight (M_w) and molecular weight polydispersity (PDI) of 1500 and 2.46, respectively, and no residual PEG200 was detected.

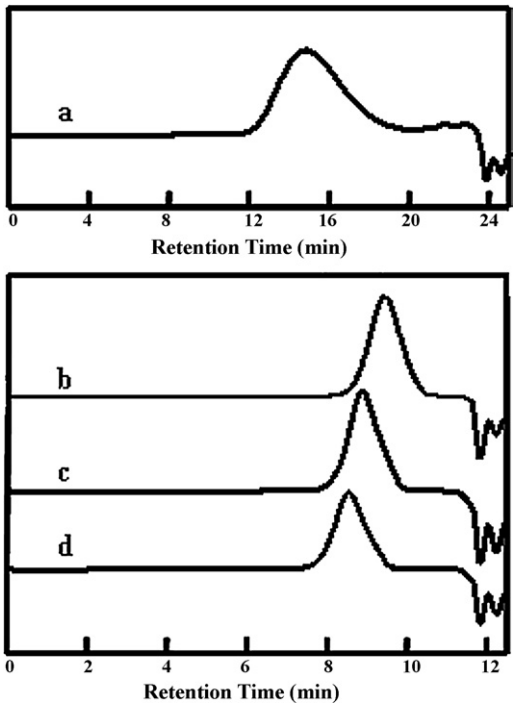


Fig. 2. GPC elution profiles of PBE (a), PBELA with PBE contents of 10% (b) and 5% (d), and PELA-10 (c).

Fig. 1b showed a representative ^{1}H NMR spectrum of PBELA as well as the detailed assignment of each peak. It indicated the methyl proton of LA at 1.5 ppm, phenyl group at 7.2 ppm and acetal group at 5.6 ppm of PBE. It should be noted that no signal at 2.3 ppm, which was assigned to the hydroxyl end groups of PBE, was detected in the spectrum of PBELA, indicating that PBE was completely involved in the copolymerization. The signal at 4.2 ppm should be attributed to the methylene protons of PBE segments close to the ester linkage of PLA blocks. The FTIR spectrum of PBELA showed that the strong absorptions at 1756 cm^{-1} (C=O stretching), 2996 cm^{-1} (C–H stretching of –CH_3 of ester segments), and 2881 cm^{-1} (C–H stretching of –CH_2–CH_2–O of PEG segments). The most characteristic absorption of C–O–C of PEG segments at 1117 cm^{-1} was overlapped by C–O stretching of ester segments. The formation of PBELA copolymer was also confirmed by GPC analysis, which gave only one peak (Fig. 2). Copolymers were obtained with M_w of 27 and 16 kDa and PDI of 1.26 and 1.31 for PBELA with PBE contents of 5% (PBELA-5) and 10% (PBELA-10), respectively. As the control to the acid-labile polymer for matrix degradation and drug release investigations, triblock copolymer PELA containing 10% of PEG2000 (PELA-10) was obtained with M_w of 25 kDa and PDI of 1.21.

3.2. Characterization of electrospun fibers

Fig. 3 showed the SEM morphologies of electrospun fibrous mats, which possessed the common feature of being bead-free, randomly arrayed, and very porous. Their surfaces were smooth and no drug crystals were detected, indicating that the drug was finely incorporated into the electrospun fibers. Average diameters of 1123 ± 114, 914 ± 92 and 1296 ± 204 nm were obtained for paracetamol loaded fibers of PELA-10, PBELA-5 and PBELA-10, respectively.

Paracetamol-loading amount was 1.96 ± 0.02%, close to the theoretic value (2.0%, w/w) for all fiber samples. To further demonstrate the physical state of paracetamol in the fibers, paracetamol, PBELA-10, and paracetamol/PBELA-10 fibrous mats were character-

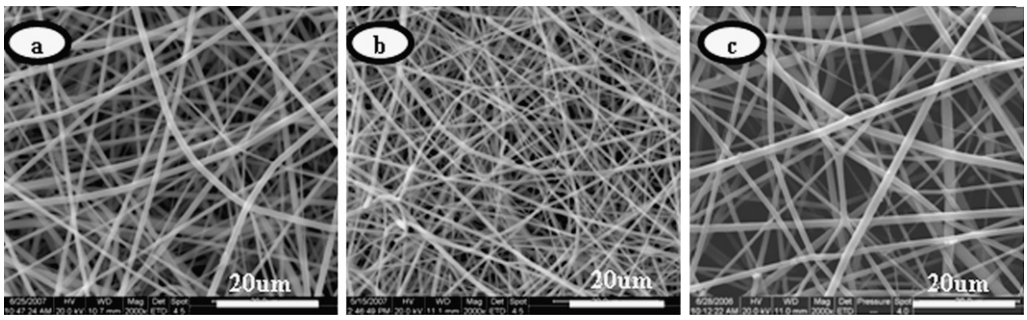


Fig. 3. SEM photographs of electrospun PELA-10 (a), PBELA-5 (b) and PBELA-10 (c) fibrous mats containing 2.0 wt% of paracetamol.

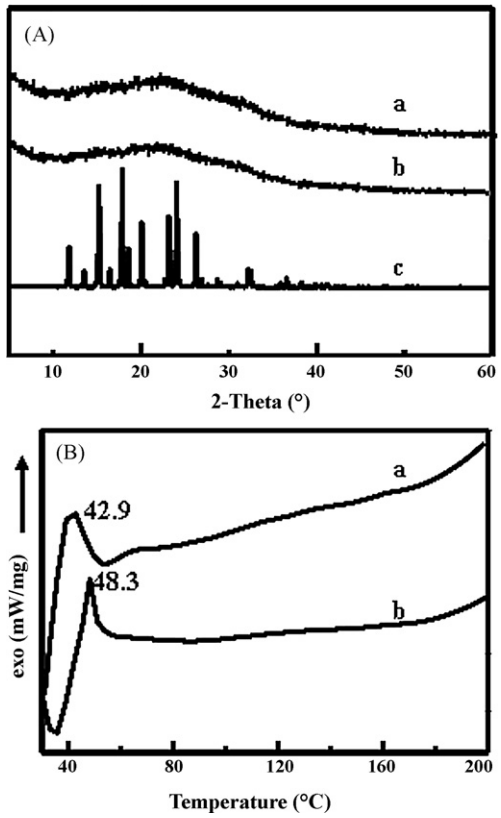


Fig. 4. (A) XRD patterns of PBELA-10, a; paracetamol/PBELA-10 fibers, b and paracetamol, c. (B) DSC patterns of paracetamol/PBELA-10, a; and PBELA-10 fibrous mats, b.

ized by XRD. As shown in Fig. 4A, pure paracetamol was crystalline with characteristic peaks at $2\theta = 15.5^\circ$, 18.2° and 24.4°, respectively. The crystalline paracetamol was not detected in all paracetamol-containing polymer fibers, suggesting that paracetamol existed in amorphous form, probably as a solid solution or amorphous molecular aggregates in polymer fibers. Fig. 4B showed DSC curves of electrospun fibrous mats and drug loaded electrospun mats of PBELA-10. By incubating drug into electrospun polymeric fibers, the small molecular drug acted among the molecular chains and made molecular chains moving easily, which led to a lower T_g of 42.9 °C. The glass transition enthalpy was 6.33 and 11.28 J/g for electrospun mats of PBELA-10 with and without drug entrapment, respectively. The drug inoculation led to irregular alignment of polymer chain, which led to a slight decrease in the transition enthalpy.

3.3. In vitro paracetamol release profiles

The drug release profile from electrospun fibers of acid-labile polymer matrices were evaluated in buffer solutions of different pH values, and the results are summarized in Fig. 5. Fig. 5a showed the paracetamol release from PELA-10 fibers mat after incubation in pH 7.4, 5.5 and 4.0 buffer solutions. Similar release behaviors were detected, and the total amounts of release were around 20, 21 and 25% for PELA-10 fibers during 120 h incubation in buffer solutions of pH 7.4, 5.5 and 4.0, respectively. Fig. 5b and c showed the release profiles from pH-sensitive polymers with different amounts of acid-labile segments under different buffer solutions. As shown in Fig. 5b, the drug release rate from PBELA-5 fibers at pH 5.5 and 4.0 became higher than that at pH 7.4, and the total amount of release was about 23, 39 and 50% after incubation for 120 h in buffer solutions of pH 7.4, 5.5 and 4.0, respectively. The differences of release profiles were more significant for PBELA-10 with higher amount of acid-labile segments between in the acid and in neutral buffer solutions (Fig. 5c). The total amount of release was about 67 and 78% after incubation in pH 5.5 and 4.0 buffer solutions for 120 h, respectively, while that was only 26% after incubation in pH 7.4.

As indicated in Fig. 5, initial burst release was observed during the initial 6–12 h for all the fiber samples incubated into medium of different pH values, followed by sustained release. The burst release was contributed by the paracetamol molecules dispersing close to

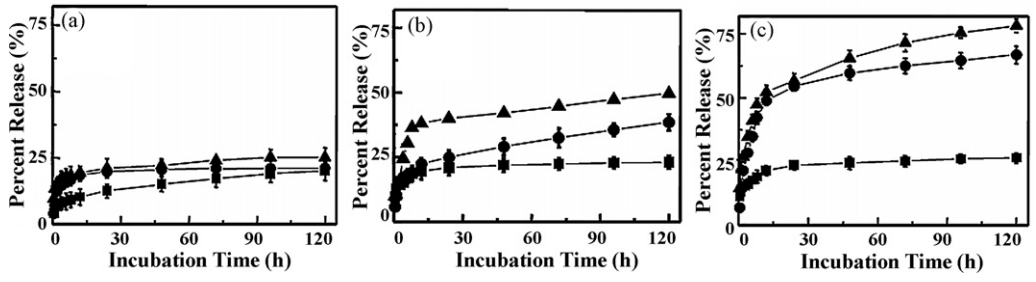


Fig. 5. Release profiles of paracetamol from PELA-10 (a), PBELA-5 (b) and PBELA-10 fibers (c) with 2.0% of paracetamol entrapment in pH 7.4 (■), 5.5 (●) and 4.0 (▲) buffer solutions at 37 °C ($n = 3$).

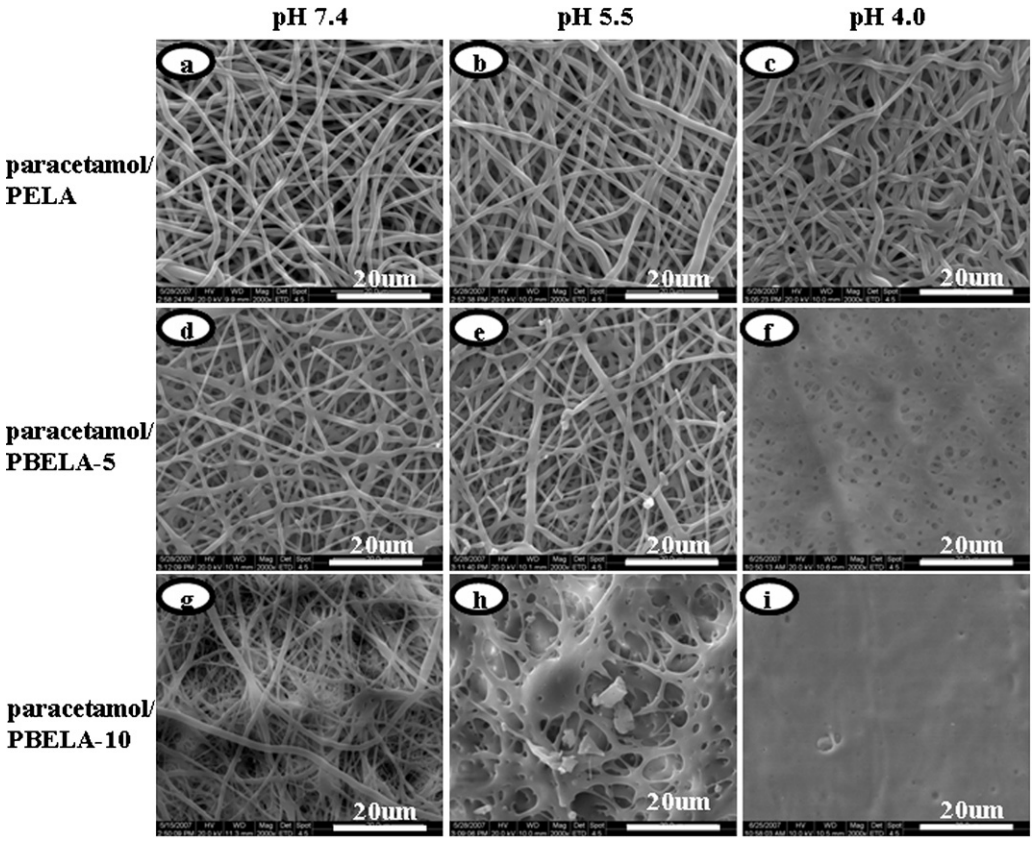


Fig. 6. SEM morphologies of electrospun paracetamol/PELA-10 fibrous mats in pH 7.4 (a), 5.5 (b) and 4.0 (c), of paracetamol/PBELA-5 mats in pH 7.4 (d), 5.5 (e) and 4.0 (f), and of paracetamol/PBELA-10 mats in pH 7.4 (g), 5.5 (h) and 4.0 buffer solutions (i) after incubation for 24 h at 37 °C.

the polymer fibers surface and adsorbing at or loosely binding near the surface, which diffused out in the initial incubation time. There was around 10% of the loading amount that initially released out for all the fiber samples after incubated in pH 7.4 buffer solution during the initial 12 h. The amounts of burst release were significant higher for PBELA with higher contents of acid-labile segments and for polymer fibers incubated in medium of lower pH values. This may be due to the erosion of fiber surface and the detachment of drug from fiber matrix under acid buffer solutions. After incubated in pH 4.0 buffer solution, around 20, 36 and 50% of the paracetamol release were detected in the initial 12 h for PELA-10, PBELA-5 and PBELA-10 fibers, respectively. The sustained release from the polymer matrix was mainly controlled by not only the drug diffusion through the matrix but also the matrix degradation (Seong et al., 2003). The porous structure of fibrous mat and the micropores after the diffusion out of drug molecules from the out layer made it possible that further constant release of drug from inner part. As shown in Fig. 5, the sustained release of paracetamol from PELA-10, PBELA-5 and PBELA-10 fibers after incubated in pH 7.4 was not as significant as those after incubated in acid buffer solutions. Amount of drug still entrapped into the fibers, which maybe would not release out until significant degradation of matrix polymer occurred (Li et al., 2001). Similar amount of drug released from three kinds of polymer fibers could be found after incubated in pH 7.4 buffer solution for 120 h. About 20, 23 and 26% of the loading amount were released from PELA-10, PBELA-5 and PBELA-10 fibers, respectively (Fig. 5). All the polymer matrices were relatively stable in pH 7.4 buffer solution during 120 h, and it was assumed that the degradation of matrix polymer was not predominant and the paracetamol release could be principally attributed to the diffusion mechanism (Ritger and Peppas, 1987). As a control to acid-labile polymer, similar sustained profiles were detected for PELA-10 fibers after incubated in different buffer solutions (Fig. 5a). However, when the pH value of buffer solutions were 5.5 and 4.0, the amount of drug released from pH-sensitive polymer fibers was accelerated on account of the pH-induced structural changes of the polymeric fibers and the degradation of matrix polymer. As shown in Fig. 5b and c, the drug release rate from PBELA-10 fibers was higher than that from PBELA-5 at pH 5.5 or 4.0. About 25, 50 and 78% of the loading amount were released from PELA-10, PBELA-5 and PBELA-10 fibers after incubated in pH 4.0 buffer solution for 120 h, respectively.

Thus, it may be concluded that the amount of initial burst release and the time period of sustained release from electrospun medicated fibers were controlled through the matrix polymer with different contents of acid-labile segments and the local pH environment. Dependent on the pathological conditions, larger initial burst release, faster 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 eliminate the intruding bacteria before they began to proliferate for inflammation control. And for those that may survive the initial burst, a continued release of antibiotic was also necessary to prevent their further population. Delayed release behaviors obtained form above investigation demonstrated a potential for programmed delivery by combination of fibers with different contents of acid-labile segment for clinical needs. The release rate and the length of sustained release can be adjusted to closely relate with the local pathological conditions.

The structure integrity of paracetamol released from electrospun fibrous mats was examined by HPLC. It showed that all released paracetamol had the same retention time at 4.7 min with-

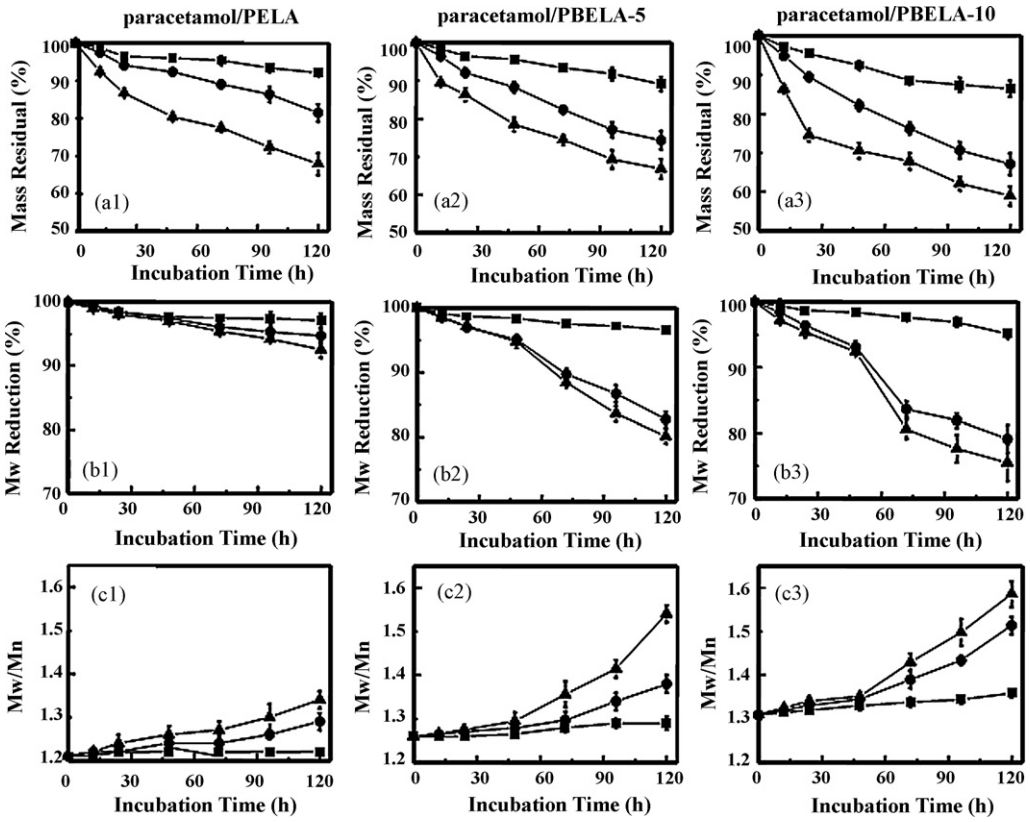


Fig. 7. The residual mass percent of fibrous mats (a), molecular weight reduction (b) and molecular weight polydispersity (M_w/M_n) of matrix residue (c) of PELA-10 (a1–c1), PBELA-5 (a2–c2) and PBELA-10 (a3–c3) fibers with 2.0 wt% paracetamol entrapment after incubation in pH 7.4 (■), 5.5 (●) and 4.0 (▲) buffer solutions at 37 °C ($n = 3$).

out new peaks appearance (data not shown). In terms of these results, it can be concluded that paracetamol almost hold the structure integrity and showed no interactions with the matrix polymer during the electrospinning process and the release period from polymeric fibers. It is essential to clarify the obtained results of drug release and matrix degradation, which were only closely related with the pH environment and the matrix polymer itself.

3.4. In vitro degradation behaviors of pH-sensitive polymers

Degradation profiles of the fibrous mats were assessed macroscopically by SEM. After incubated into degradation medium, the non-woven fibrous mat floated, then suspended and immersed into the medium. Non-woven mat changed from shrinking to puffing bigger than previously, meanwhile, fibers size increased and fibers space shrunk for all samples (Cui et al., 2006). It was observed that electrospun PELA-10 fibrous mat kept the fibers morphologies after incubation in buffer solutions of pH 7.4, 5.5 and 4.0 for 120 h. Meanwhile, similar fibers morphologies were found for PBELA-5 and PBELA-10 fibrous mats after incubation in pH 7.4 buffer solution. But after incubation in buffer solutions of pH 5.5 and 4.0, electrospun PBELA-5 and PBELA-10 fibers became flatter and collapsed from their previous cylindrical shape, and gradually formed polymeric films without evident fiber morphologies. Significant morphological changes were observed for PBELA-5 and PBELA-10 fibrous mats after incubation for 48 and 24 h in pH 5.5 buffer solution, and for 24 and 12 h in pH 4.0, respectively. Fig. 6 showed the morphologies of electrospun PELA-10 (Fig. 6a–c), PBELA-5 (Fig. 6d–f) and PBELA-10 (Fig. 6g–i) fibrous mats at 24 h after incubation in different media. Electrospun PELA-10 fibers were swollen (Fig. 6a–c) and curly compared with original formation (Fig. 3a), due to the chain relaxation of matrix polymer after incubated into the medium with elevated temperature. And the shrinkage and congeries of fibers could be easily found for PBELA-5 and PBELA-10 fibers after incubation for 24 h in buffer solutions of pH 7.4 (Fig. 6d and g) and 5.5 (Fig. 6e). Morphological coexistence of fibers and film could be found for electrospun PBELA-5 mats incubated in pH 4.0 buffer solution (Fig. 6f) and for PBELA-10 mats in pH 5.5 (Fig. 6h). Higher sensitivity of fibers morphology to lower pH environment was observed for PBELA-10 fibrous mats, and polymeric films without evident fiber morphologies were detected for PBELA-10 mats incubated in pH 4.0 buffer solution (Fig. 6i).

The degradation behaviors of paracetamol loaded PELA-10, PBELA-5 and PBELA-10 fibers were determined in buffer solutions of different pH values with regard to the mass loss of the fibrous mats, molecular weight reduction and molecular weight polydispersity of the matrix polymer. Gravimetric evaluation of the mass loss during incubation was summarized in Fig. 7a. There were about 8, 19 and 32% of mass loss for electrospun PELA-10 fibrous mats after incubation for 120 h in buffer solutions of pH 7.4, 5.5 and 4.0, respectively (Fig. 7a1). The mass loss of the fibrous mats may be resulted from the dissolve 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 11, 16 and 33% of mass loss for PBELA-5 (Fig. 7a2) and 14, 33 and 41% of mass loss for PBELA-10 (Fig. 7a3) were detected in buffer solutions of pH 7.4, 5.5 and 4.0, respectively. Fig. 7b showed the molecular weight reduction of matrix polymer of fibrous mats. Less than 10% of molecular weight reduction was found for PELA-10 fibrous mat in three kinds of buffer solutions, and similar results of PBELA-5 and PBELA-10 mats could be found in pH 7.4 buffer solution. The molecular weight decreased gradually with incubation time and no significant difference was found for PBELA-5 and PBELA-10 mats after incubation into pH 7.4 buffer solution. There

were around 18 and 20% of molecular weight loss for PBELA-5 mats, and 21 and 25% for PBELA-10 after incubation for 120 h in pH 5.5 and 4.0 buffer solutions, respectively. An increase in polydispersity was observed for all samples (Fig. 7c), paracetamol loaded PELA-10 electrospun fibers showed approximately 0.8, 6.6 and 10.7% of increase after incubation for 120 h in pH 7.4, 5.5 and 4.0 buffer solutions, respectively. Significant larger molecular weight distribution was detected for matrix polymer with inclusion of acid-labile segments. And there were 2.4, 9.5 and 22.2% of increase for PBELA-5, 3.7, 15.3 and 21.1% of increase for PBELA-10 after incubation in pH 7.4, 5.5 and 4.0 buffer solutions, respectively.The degradation behavior of matrix polymer is one of the most important aspects for the end application as drug carriers and tissue growth scaffolds. As shown in Fig. 6, the morphological changes of the fibrous mats to collapsed film type led to a significant decrease in effective surface area for drug release, which may cause a delayed release. On the other hand, the breakdown of the polymer backbone and the mass loss of the fibrous mat, as shown in Fig. 7, would enhance the diffusion of drug out of the fiber matrix. 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. 5.The processes involved in the erosion of a degradable polymeric fiber are complicated. 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 type of chemical bond, compositions and water uptake are the most important factors that influence the pattern and velocity of degradation process. As shown in Fig. 7a1 and b1, limited mass loss and molecular weight reduction were observed for electrospun PELA-10 fibers mat after incubation for 120 h in pH 7.4 buffer solution. But mass loss was about 32% for PELA-10 mats during 120 h incubation in pH 4.0 buffer solution, while molecular weight loss of 8.6% was obtained. The mass loss was significantly higher than the molecular weight reduction, which showed a typical surface erosion pattern due to the hydrophobic surface of electrospun fibrous mats (Cui et al., 2006). The high water repellent property of electrospun fiber led to much slower water penetration into the fibrous mat, which may be attributed to the degradation profiles of surface erosion.

As shown in Fig. 7b2 and b3, the degradation behaviors of PBELA-5 and PBELA-10 fibrous mats in pH 7.4 buffer solution were close to PELA-10 mats, while significant higher mass loss and molecular weight reduction were detected after incubation into acidic buffer solutions. It was indicated that the electrospun fibers of PBELA containing acid-labile segments were as "stable" as PELA-10 without acid-labile segments under neutral buffer solution, but highly sensitive to the acid environment. The acid-labile segment of PBELA 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 (Fig. 7c). Meanwhile, a turning point of molecular weight reduction could be found at 48 h for PBELA-5 and PBELA-10 incubated in pH 5.5 and 4.0 buffer solutions (Fig. 7b2 and b3), and significant higher molecular weight reduction rates of the matrix polymers were found after the point. As shown in Fig. 6, morphological changes of electrospun PBELA-5 and PBELA-10 fibrous mats into polymeric film were observed after incubation into pH 5.5 and 4.0 buffer solutions for 24–48 h, which should determine the degradation behaviors. The degradation process of pH-sensitive polymer fibers of PBELA in acid solutions could be divided into two stages. The first stage was the degradation of electrospun fibers. For PBELA-5 electrospun fibrous mats incubated in pH 4.0 buffer solution for 48 h, there were 5.2% of molecular weight reduction and 21.5% of mass loss. And PBELA-10 mat showed 7.6 and 29.5% of molecular weight reduction and mass loss, respectively. Obviously these were not consistent with bulk degradation of common formulations (Burkersroda et al., 1997), and seemed more likely the surface erosion mechanism (Burkersroda et al., 2002). While incubated in acid buffer solutions, the pH-sensitive polymer of PBELA hydrolyzed from the fiber surface, transformed into soluble fragments, leading to significant mass loss. To clarify the degradation profiles of surface erosion, the matrix polymer of fibrous residue was characterized using ^{1}H NMR and FTIR (data not shown), which did not display any shifts in the peak position and indicated that the chain scission did not occur at the polyether segments of residual polymers during the initial incubation period.

The second degradation stage of PBELA in acidic buffer solution was characterized by faster molecular weight reduction and slower mass loss compared to the initial 48 h. There were 11.7% of mass loss and 14.7% of molecular weight reduction for PBELA-5 fibrous mats incubated in pH 4.0 buffer solution from 48 to 120 h. And PBELA-10 mats showed 11.6 and 16.9% of mass loss and molecular weight reduction, respectively. The molecular weight reduction went beyond the mass loss of the matrix polymers, thus, the hydrolysis of polymer films should proceed through the bulk of polymer structure, which was in good agreement with the bulk degradation mechanism (Burkersroda et al., 2002). Therefore, when the pH-sensitive polymer of PBELA electrospun fibrous mats was incubated into acid buffer solution, there were a two-stage degradation pattern of initial surface erosion for electrospun fibrous mats with distinct fiber morphologies followed by bulk degradation for polymeric film resulting from the morphological changes during the followed incubation.

4. Conclusions

A novel strategy was developed to synthesize acid-labile polymers by incorporating acetal groups into biodegradable backbone. *In vitro* release study showed that the amount of initial burst release and the time period of sustained release from electrospun medicated fibers were controlled through the matrix polymer with different contents of acid-labile segments and the local acid environment. During the investigational period, the acid-labile polymer was stable in neutral buffer solutions, and the degradation was enhanced in acid buffers, showing a two-stage degradation profile. It is suggested that acid-labile fibrous mats be potential drug carriers, and the matrix degradation and drug release profiles were responded to local acid environment and the acid-labile components of the matrix polymer.

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