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Dual-responsive drug delivery systems prepared by blend electrospinning

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Abstract:

To prepare temperature and pH dual-responsive drug delivery systems, the thermosensitive polymer poly(N-isopropylacrylamide) (PNIPAAm) was first synthesized by free-radical polymerization. It was then co-dissolved with the pH-sensitive polymer Eudragit® L 100-55 (EL100-55) and processed into fibers using electrospinning. Ketoprofen (KET), a model drug, was also incorporated into the composite fibers, and fibers based on a single polymer additionally prepared. The fibers had smooth cylindrical morphologies, and no obvious phase separation could be seen. Using X-ray diffraction, KET was determined to be present in the amorphous state in the fiber matrix. FTIR spectroscopy also indicated the successful incorporation of amorphous KET in the fibers. *In vitro* drug release studies in media at different pH (4.5 or 7.4) or temperature (25 and 37 ºC) showed that the release of KET from the blend PNIPAAm/EL100-55 fibers was dependent both on environmental temperature and pH, reflecting the dual-responsive properties of the fibers. The MTT assay was used to explore the biocompatibility of the PNIPAAm/EL100-55 composite fibers towards L929 fibroblasts. Viability was always found to be > 80 %, even at polymer concentrations of 100 mg/mL. Therefore, the fibers prepared here could lead to the development of multi-responsive fibers for drug

delivery and tissue engineering.

Keywords: Drug delivery system, dual-responsive, electrospinning, poly(N-isopropylacrylamide), Eudragit® L 100-55.

1. Introduction:

In recent years, electrospun fiber-based drug delivery systems (DDSs) have drawn great attention from the research community. Electrospinning, being a relatively simple and cost-effective technique, has become a commonly used method to produce non-woven nanoscale fibers [1, 2]. The resultant mats have several intrinsic benefits including high porosity, tunability, a high surface area-to-volume ratio, and a similar structure to the extracellular matrix in tissues. Electrospun fibers can also have high (> 50%) drug loadings and good biocompatibility, important properties for potent DDSs [3-8]. By careful selection of the drug loading modality and the material used to develop the fibers, the drug release rates can be tuned [9]. Different active ingredients can be loaded into the fibers, and the drug location in the polymer matrix controlled [10]. In addition, a large number of polymers (ca. 100) have been successfully fabricated into electrospun fibers. This includes thermosensitive and pH sensitive polymers.

Poly(N-isopropylacrylamide) (PNIPAAm) is one of the most commonly explored temperature-sensitive polymers, and undergoes a rapid phase transition at a lower critical solution temperature (LCST) of 32 ºC in aqueous solution. When the temperature is increased through the LCST, PNIPAAm changes abruptly from being hydrophilic to hydrophobic [11-13]. PNIPAAm has been successfully electrospun into fibers, and also studied in the context of drug delivery [14-15]. For instance, in our previous work DDSs based on PNIPAAm and ethyl cellulose (EC) were prepared by both blend electrospinning and coaxial electrospinning; both systems had temperature-dependent drug release properties. [16-17].

The Eudragit family of polymers are well known pharmaceutical excipients developed by the Röhm Company in Germany. They are based on polymethacrylates and have been widely used in the preparation of extended and delayed release formulations. Eudragit L100-55 (EL 100-55) (methacrylic acid - ethyl acrylate copolymer type A, 1:1 molar ratio), is a pH-dependent copolymer which is highly soluble in water above pH 5.5, but insoluble below this pH. To date, EL 100-55 has been extensively used for the formulation of dosage forms including as enteric tablet coatings, microspheres, nanoparticles and fibers. These systems have been investigated for various applications, particularly colon-targeted drug delivery [18-20]. For instance, EL100-55 fibers loaded with diclofenac sodium (DS) were successfully prepared using an electrospinning process and demonstrated to possess pH-dependent drug release profiles [21].

DDSs based on responsive polymers could have a number of promising drug delivery properties, and hence such "smart" polymers have explored extensively [22], including in the preparation of DDSs responsive to more than one stimulus. To date, a number of studies into dual-responsive DDSs using temperature and pH responsive polymers have been reported [23-27]. For example, Li et al. developed a series of biodegradable multi-sensitive poly(ether-urethane)s [28]. An aqueous solution of these multi-segment copolymers underwent a sol-to-gel phase transition with increasing temperature and pH. In addition, the controlled release of insulin could be achieved [28]. In other work, Chen et al. synthesized a series of dual-responsive block copolymers containing poly(ε-caprolactone) and poly(triethylene glycol) units, which formed micellar structures in aqueous solutions. The 6-aminocaproic acid-functionalized copolymer possessed pH-sensitive phase transitions at mildly acidic pHs and body temperature. Doxorubicin-loaded micelles prepared from this polymer showed increased drug release at acidic pH, and more potent anti-cancer activity than free doxorubicin in mice [29]. To date, most reports of temperature and pH dual-responsive DDS are based on hydrogels and self-assembled micelles. Further, they tend to rely on the generation of new copolymers, which often means that the

preparation of these materials is complicated and time consuming.

In this work, we adopt a more straightforward approach by simply combining a pH-sensitive and a thermoresponsive polymer. PNIPAAm was employed as the latter, and EL100-55 the pH-sensitive material. Through blend electrospinning, PNIPAAm/EL100-55 composite fibers could be produced. Ketoprofen (KET), a non-steroidal anti-inflammatory drug, was selected as a model active ingredient and incorporated into the composite fibers to form a dual-responsive drug delivery system. Morphological and physicochemical characterizations, *in vitro* drug release, and biocompatibility assays were undertaken.

2. Experimental:

2.1 Materials

N-isopropylacrylamide (NIPAAm) was procured from Japan TCI (Japan). Phosphate-buffered saline (PBS), sodium azide, penicillin, trypsin and thiazolyl blue (MTT) were purchased from Sigma-Aldrich Ltd. (USA). Azobisisobutyronitrile (AIBN), anhydrous ethanol, acetone, formaldehyde and n-hexane were provided by the Sinopharm Chemical Reagent Co., Ltd (China). Eudragit L 100-55 (average molecular weight approximately 135,000 Da) was supplied by Rohm GmbH (Germany). Ketoprofen (KET) was procured from Beijing J&K Scientific Co., Ltd. (China). L929 cells were obtained from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, China). Dimethyl sulfoxide (DMSO) and DMEM culture medium were sourced from Jinuo Biological Medicine Technology Ltd. (China). All other chemicals used were analytical grade, and water was doubly distilled before use.

2.2 PNIPAAm synthesis

Free-radical polymerization was used to synthesize PNIPAAm, following protocols used in previous work [30]. 5.0 g NIPAAm and 25.0 mg AIBN were dissolved in 10 mL anhydrous ethanol, and the reaction system heated at 70 ºC under a positive

pressure of N_2 for 7 hours. The products were then precipitated in n-hexane. The crude product was subsequently re-precipitated from 50 mL of acetone into 200 mL of n-hexane three times, and the purified material dried for 3 days in a vacuum oven (DZF-6050, Shanghai Laboratory Instrument Work Co. Ltd., China). Successful polymerization was confirmed by ^{1}H nuclear magnetic resonance (AV-400 instrument, Bruker, Germany) and IR spectroscopy (Nicolet-Nexus 670 FTIR spectrometer, Nicolet Instrument Corporation, USA). Molecular weights (Mw, Mn) and molecular weight distributions were tested by gel permeation chromatography (GPC) measurements on a Waters LS measurement system (Waters, USA) with tetrahydrofuran (THF) as the solvent. The flow rate was 1.0 mL/min, and the column temperature was 35 ºC. The molecular weight distribution was calibrated with standard polystyrene samples.

2.3 Preparation of electrospinning solutions

PNIPAAm and EL100-55 were dissolved into ethanol under magnetic stirring for 8 h at room temperature to yield clear and homogenous solutions. The component ratio of PNIPAAm to EL100-55 was 1:1 (w:w), and the total concentration of polymer 20 % (w/v). Solutions of PNIPAAm and EL100-55 alone (20 % w/v in ethanol) were also prepared as controls. The model drug KET was added into selected solutions at a drug to polymer ratio of 1:5 (w:w). Full details of the solutions prepared are listed in Table 1.

Table 1. Details of the electrospinning solutions prepared in this work.

Sample	Solution contents	PNIPAAm to EL100-55 ratio (w/w)	KET concentration (% w/v)
S1	PNIPAAm/EL100-55	1:1	---
S2	PNIPAAm	---	---
S3	EL100-55	---	---
S4	PNIPAAm/EL100-55	1:1	4.0
S5	PNIPAAm	---	4.0
S6	EL100-55	---	4.0

2.4 Electrospinning

The required solution was loaded into a 5.0 mL plastic syringe, which was fitted with a stainless steel needle (internal diameter 0.5 mm) and placed on a syringe pump (KDS100, Cole-Parmer, USA). A flow rate of 1.0 mL/h was used for electrospinning, with a voltage of 16 kV applied (ZGF-2000 power supply, Shanghai Sute Electrical Co. Ltd., China). The distance between the grounded collector and needle tip was 20 cm. Spinning was conducted at ca. 40 % relative humidity, and at room temperature (25 ºC). As soon as the solution in the syringe was finished, the syringe was refilled immediately. Each experiment was performed for 8 h, after which the fiber mats were produced were removed from the collector and stored in a vacuum oven at room temperature for 24 h to remove any residual solvent.

2.5 Fiber characterization

The fibers were imaged using a scanning electron microscope (SEM; JSM-5600 LV microscope, JEOL, Japan) at a voltage of 10 kV. Prior to scanning, the samples were gold sputter-coated under argon for 60 s to make them electrically conductive. The ImageJ software (National Institutes of Health, USA) was used to calculate the average fiber diameter of each sample by analyzing ca. 100 fibers in each of the SEM images. Cross-sections of the nanofibers were prepared by immersing them into liquid

nitrogen and then manually breaking the mats.

X-ray diffraction (XRD) was undertaken on a D/Max-BR diffractometer (Rigaku, Japan), which was supplied with Cu Kα radiation (40 kV / 30 mA). Patterns were collected over the 2θ range 5–60º. Fourier transform infrared spectroscopy (FTIR) data were obtained using a Nicolet-Nexus 670 FTIR spectrometer (Nicolet Instrument Corporation, USA). Spectra were acquired over the range 500–4000 cm^{-1} at a resolution of 2 cm^{-1}.

2.6 *In vitro* drug release

Drug release experiments were conducted at two different temperatures (25 or 37 ºC) and in media at two different pH values (pH 7.4 PBS and pH 4.5 acetic acid buffer) at 110 rpm in a thermostatic shaking incubator (Jintan Instrument Co. Ltd., China). 50 mg of each fiber mat was separately immersed in 30 mL of a release medium. At predetermined time points, 1 mL of the test medium was withdrawn and an equal amount of fresh pre-heated medium added. The amount of KET released was determined using a UV-vis spectrometer (UV-1800, SHJH Company, China) at a wavelength of 265 nm, following construction of a calibration curve. All release studies were performed in triplicate, and the results are given as mean ± S.D.

2.7 Cytotoxicity measurements

The *in vitro* cytotoxicity of samples was investigated with the aid of MTT assays. L929 fibroblasts were employed and cultured in DMEM medium supplemented with 10 % v/v FBS and 1 % v/v penicillin–streptomycin solution. For cell viability measurements, 200 μL of cell suspension was seeded into 96-well plates at a density of 10^4 cells/well. After incubation (37 ºC, 5 % CO_2) overnight, the medium was removed and fresh medium containing different concentrations (100, 50, 25 mg/L) of fibers S4, S5 or S6 was added. Blank DMEM was used as a negative control. After incubation for another 24 hours, the culture medium in each well was replaced by 180 μL of fresh DMEM and 20 μL of an MTT solution (5 mg/mL thiazolyl blue in PBS).

The plates were shaken for 30 min at room temperature, before 200 μL DMSO was added to each well and the plates incubated for 6 h. The resultant purple solution in each well was used to determine the number of cells, which was assessed via the OD values at 570 nm. The latter were quantified on a microplate reader (Multiskan, ThermoFisher, USA). The relative cell viability was measured by comparing the treatment wells to the control containing the untreated cells in DMEM (deemed to have 100% viability).

3. Results and discussion:

3.1 PNIPAAm synthesis

Successful polymerization of NIPAAm was verified by ^{1}H NMR data (Fig. 1). The polymer (Fig. 1(a) displays no methylene protons, while these are clearly visible for the monomer (NIPAAm, Fig. 1(b)) at ca. 5.5 and 6.3 ppm. Instead, PNIPAAm has signals at around 1.5 and 2.1 ppm. These are typical of saturated systems and agree with a previous study [30], indicating the successful formation of PNIPAAm. FTIR spectra (Fig. 2) confirm the successful synthesis of PNIPAAm. There is a sharp band at 1621 cm^{-1} (C=C stretching vibration) in the spectrum of NIPAAm, which disappears after polymerization. GPC characterization determined that the molecular weights (Mw and Mn) and molecular weight distribution (PDI) of the PNIPAAm product were 50,052, 63,899, and 1.67, respectively.

Fig. 1

Fig. 2

3.2 Fiber morphology

SEM images and the diameter distributions for the electrospun products (S1-S6) are shown in Fig. 3. The images confirm that regular cylindrical fibers have been successfully produced under all the conditions used in this study. The blend fibers S1 have larger average diameter (1116 ± 290 nm) than pure PNIPAAm (S2, 891 ± 188

nm) and EL100-55 (S3, 976 ± 208 nm) fibers. With the addition of KET, the average diameters of the fibers increased: the average diameters of S4, S5 and S6 are 1396 ± 284 nm, 1097 ± 225 nm and 1015 ± 212 nm, respectively. A cross-sectional image of the S4 fiber mat was also obtained (see Fig. S1, Supporting Information): this shows the fiber cross-sections are smooth and homogeneous. There is no phase separation evident, suggesting an even dispersion of drug in the polymer carrier.

Fig. 3

3.3 X-ray diffraction

XRD patterns of pure KET and all the fibers are given in Fig.4. It is apparent that for KET there are a number of characteristic reflections at 6.5°, 13.2°, 14.0°, 18.0°, 20.2°, 21.5°, 23.0°, 23.8°, 26.5° and 29.8° 2θ, indicating the crystalline nature of the pure drug. This is consistent with the literature [31]. In contrast, none of the fibers show any Bragg reflections. S2 and S5 (PNIPAAm based) display two weak and broad diffuse peaks at around 8º and 23º 2θ in their patterns, while the other formulations have no distinct features. The fibers are thus all amorphous materials. The characteristic reflections of KET do not appear in the KET-loaded fibers (S4-S6), demonstrating that these systems comprise amorphous solid dispersions. Similar results have been reported in many previous studies in the literature [32-34]. The formation of amorphous products can be attributed to the rapid solvent evaporation which occurs in electrospinning. As a result, there is insufficient drying time for the molecular organization required to form a crystal lattice [35, 36]. The random arrangement of molecules in the solution phase is thus propagated into the solid state, resulting in a molecular dispersion of KET in the fibers.

Fig. 4

3.4 FTIR spectroscopy

FITR spectra of pure KET and the fibers (S1-S6) are presented in Fig. 5. Neat PNIPAAm fibers (S2) display a broad band between around 3700 and 3000 cm^{-1}, which corresponds to H-bonded N-H stretches (3284 cm^{-1}) and amide I and II combination and overtone bands [37]. Two characteristic peaks at 2967 cm^{-1} and 1644 cm^{-1} result from C-H and C=O stretching vibrations, respectively. The distinct band at 1538 cm^{-1} arises from C-N stretching and N-H bending vibrations. The pure Eudragit L100-55 fibers (S3) exhibit a broad band characteristic of hydroxyl groups (O-H stretching) from 3700 to 2400 cm^{-1}. They also show methyl and methylene C-H stretching vibrations at 2998 cm^{-1} and 2930 cm^{-1}. A strong band from carbonyl groups is present at 1731 cm^{-1}, and two bands from ester linkages at 1278 and 1184 cm^{-1} can also be seen [21]. The drug free composite fibers (S1) have two distinct peaks at 1638 and 1725 cm^{-1} arising from C=O stretching in PNIPAAm and the C-O stretch of EL100-55, respectively. This confirms the presence of both polymers in the blend fibers.

KET has two key peaks at 1693 and 1656 cm^{-1}, representing the stretching vibrations of its carboxylic acid group and ketone group respectively. The peak at 1654 cm^{-1} can also be observed in the drug-loaded EL100-55 fibers (S6), confirming the incorporation of KET into S6. For S4 and S5, the characteristic peaks at 1636 (S4) and 1644 cm^{-1} (S5) have been enhanced compared with the drug free analogues S1 and S2, again ratifying the presence of KET in the fibers. However, the peak at 1693 cm^{-1} cannot be seen in the drug-loaded fibers. This can be explained by the phase transformation which KET undergoes during the process of electrospinning. In its crystalline form, KET molecules are bound together in dimers through intermolecular hydrogen bonds, resulting in the appearance of the distinct peak at ca. 1693 cm^{-1} [38, 39]. The absence of this band in the KET-loaded samples suggests a lack of dimers and crystalline structure, which is accordance with the results from XRD. KET is also known to show a triplet at ca. 704 cm^{-1} when crystalline and doublet at this position when it is in the amorphous form. The triplet can clearly be seen for the raw KET material. The low intensity of these peaks in the fibers' spectra makes these peaks a

little hard to see, but on close inspection it appears that only a doublet is present. This is again consistent with KET being molecularly dispersed throughout the polymer carriers.

Fig. 5

3.5 Drug release

The results of *in vitro* release studies are presented in Fig. 6. Experiments were performed in two different media (PBS and acetic acid buffer) at two temperatures, 25 and 37 ºC.

Fig. 6

For the EL100-55/KET fibers (S6), the release behavior looks similar at both 25 and 37 ºC, but there are distinct differences between the profiles seen in the two different media. In PBS (pH 7.4), the fibers fully dissolved after about 10 h, with more than 80 % of the KET loading released. By the end of the experiment (100 h), the cumulative release of KET from the S6 EL100-55/KET fibers at 25 and 37 ºC reached similar values of 91.5 % and 92.3 %, respectively. However, S6 showed lower release rates in AA compared to PBS. These observations arise because EL 100-55 is only soluble above pH 5.5. In AA media (pH 4.5), EL100-55 is insoluble, resulting in 75.3 % and 72.9 % of KET being released at the end of the 25 and 37 ºC experiments, respectively. While it is possible that a small amount of the KET in the spinning solution might have been lost during electrospinning, this is expected to be very minimal (no more than 5 % at most). Thus, where the fibers show distinctly less than 100 % release it is believed that this is a result of some of the KET being encapsulated deep inside the fibers (and thus not diffusing to the surface during the timescale of the experiment).

The solubility of EL100-55 is controlled by the extent of ionization of the COOH groups in the polymer [40]. Drug release from matrix drug delivery systems such as

those produced here is typically controlled by diffusion, dissolution, or a combination of the two [41]. In AA, diffusion will be the rate limiting step to KET release from S6, because EL100-55 is insoluble in this medium: thus, the only way the embedded drug can escape into solution is to diffuse through the carrier. However, EL100-55 is readily soluble in PBS, and thus polymer dissolution is expected to be relatively rapid. This allows accelerated release, and also a greater percentage of the incorporated drug to be freed into solution.

For the PNIPAAm/KET (S5) fibers, the release behavior differed with temperature. At 25 ºC, around 70 % of the drug loading was released in PBS in the first 10 h and 83.1% at 100 h. At 37 ºC, after an initial burst release of 35 % in the first 10 h, the fibers showed gradual release over about 90 hours and reach only 57.5 % at the end of the experiment. The results in AA are very similar. In this case, when the temperature is below the LCST of PNIPAAm (32 ºC), the C=O and N-H groups in the PNIPAAm chains will interact with water molecules to form intermolecular hydrogen bonds [42]. As a result, PNIPAAm has good wettability, leading to rapid release. When the temperature is above the LCST, the C=O and N-H groups form intramolecular hydrogen bonds, resulting in a globular hydrophobic polymer matrix and slower release [30].

The composite S4 fibers containing both EL100-55 and PNIPAAm with KET combine the benefits of both temperature and pH responsiveness (Fig. 6), with the PNIPAAm leading to a slowing of release at 37 ºC and the EL100-55 slowing it at pH 4.5. Release is thus fastest in 25 ºC PBS and slowest in 37 ºC AA. The temperature-responsiveness of the system is particularly marked at pH 4.5, with the difference between release at 25 and 37 ºC less noticeable at pH 7.4. Clearly, drug release from S4 is sensitive to both pH and temperature. The release mechanism is thus expected to combine both that of S5 and that of S6, with diffusion of the drug dominating when the polymers are insoluble (in AA at 37 °C), dissolution when both PNIPAAm and EL100-55 are soluble (in PBS at 25 ºC). When only one polymer is

soluble (in AA at 25 ºC, or PBS at 37 ºC), we suggest that both dissolution and diffusion will be important. The soluble polymer will dissolve freely, taking drug associated with it into solution. This dissolution will leave pores in the fibers, facilitating the diffusion of drug associated with insoluble polymer into solution.

This system has potential clinical benefit in several fields, such as hemodialysis. Hemodialysis patients are vulnerable to catheter-related bloodstream infection (CRBSI), a life-threatening condition. This is because they are implanted with catheters which remain in place for a long period of time and accessed frequently, leading to frequent opportunities for infection. This is particularly problematic because many hemodialysis patients have compromised immune systems. The estimated number of CRBSI cases is approximately 250,000 annually in the United States, with an incidence of *ca.* 1.65 infections per 1000 central line days (the total number of days patients have a central line in place). In Asia, the infection rate has been reported to be of 6.8 infections per 1000 central line days [43, 44]. An antimicrobial-loaded drug delivery system with the same dual-responsive nature as those prepared in this work (i.e. where drug release is accelerated at temperatures below the usual physiological temperature, and at neutral pHs) could be employed in the catheter to inhibit the growth of microorganisms. Catheters usually operate outside the body, at temperatures below 37 ºC, and thus when the catheter is in use drug release will be more rapid than when it is between uses. A low-level drug release rate will continue during the gaps between treatments, however. Further, when blood (pH>7.0) is running through the catheter, the drug release rate will be further enhanced due to the elevated pH. Thus, it is believed that such dual-responsive materials have great potential in designing novel targeted drug delivery systems.

3.6 Cytotoxicity

The cytotoxicity of solutions of the drug-loaded fibers (S4-S6) to L929 was investigated using the MTT assay. As shown in Fig. 7, no obvious toxicity was observed for S4, S5 and S6, even at high concentrations. At final concentrations of

100 mg/L, the cell viability was 82.7 ± 3.9 % (S4), 81.6 ± 4.3 % (S5) and 85.7 ± 4.4 % (S6), indicating that the fibers have very high biocompatibility. This reflects the non-toxic nature of both polymers as reported in previous work [16, 30]. The data for S5 (PNIPAAm/KET) show slightly lower viability that S4 and S6, because PNIPAAm shows slight toxicity to cells, as has been noted previously [16]. In contrast, EL100-55 is FDA-approved for the formulation of oral dosage forms and has extremely high biocompatibility. Overall, the blend PNIPAAm/EL100-55 fibers did not show cytotoxicity to L929 cells, indicating their great potential as dual-responsive drug carriers.

Fig 7.

4. Conclusions:

In this work, the thermo-responsive polymer poly(N-isopropylacrylamide) (PNIPAAm) was first synthesized by free-radical polymerization. Fibers containing both PNIPAAm and Eudragit® L 100-55 (EL100-55) were then prepared by blend electrospinning, with both blank polymer fibers and those loaded with the model drug ketoprofen (KET) generated. Control fibers containing a single polymer were also produced. SEM images showed that all the fibers have cylindrical morphologies, with average diameters lying between 896 and 1396 nm. There was a tendency for diameter to increase with the addition of KET to the formulations. X-ray diffraction and infrared spectroscopy revealed that KET was present in the amorphous state in the fibers. *In vitro* drug release studies at different temperatures and pH showed that the KET release profiles are dependent on both temperature and pH for the PNIPAAm/EL100-55 composite fibers. Further, the fibers are highly biocompatible with the L929 cell line. PNIPAAm/EL100-55 fibers thus have potential as effective and biocompatible dual-responsive systems for drug delivery.

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Figures

Dual-responsive drug delivery systems prepared by blend electrospinning

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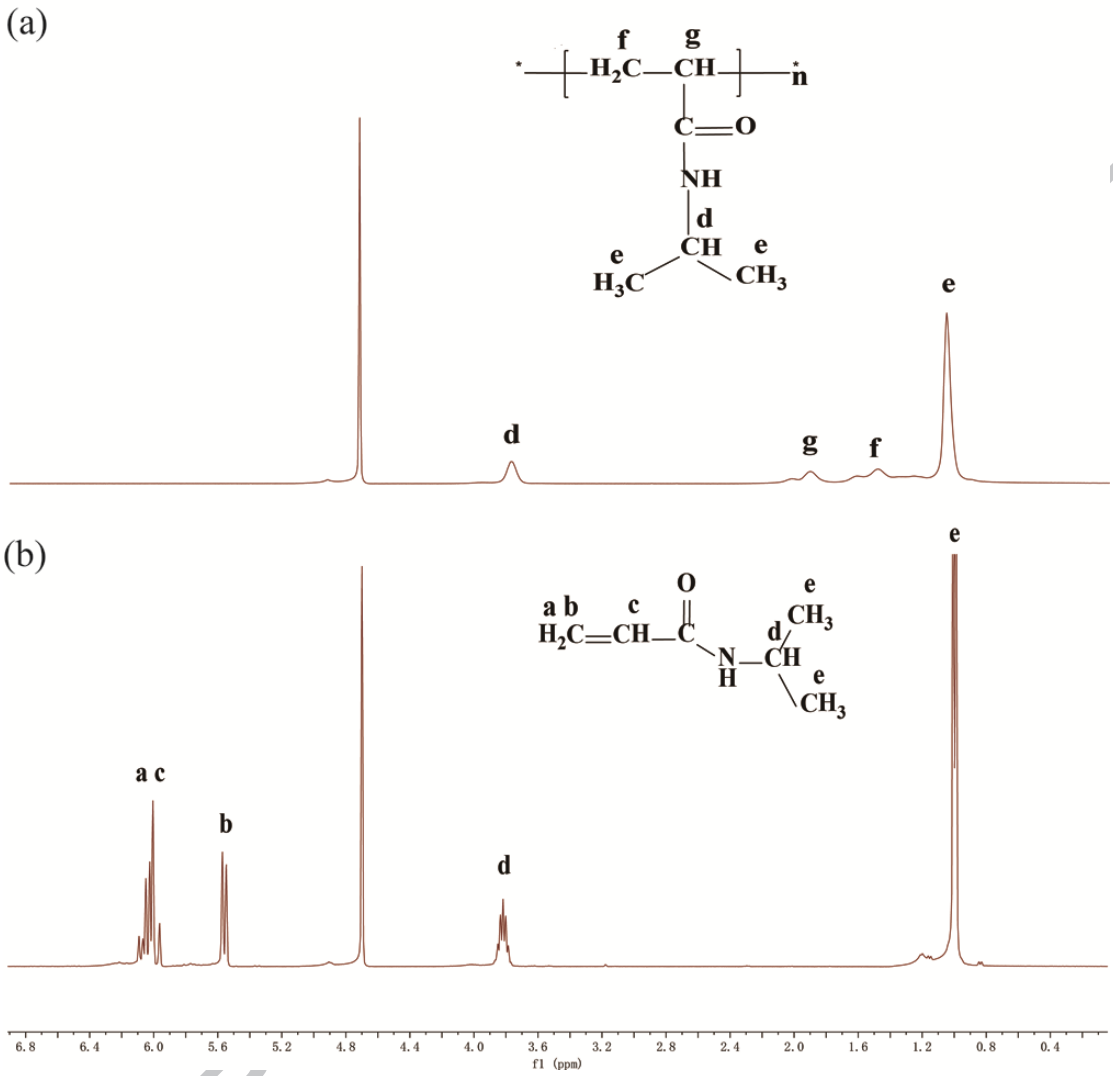

Fig. 1 ^{1}H NMR spectra of (a) PNIPAAm and (b) NIPAAm. The solvent peak (H_2O) appears at 4.7 ppm.

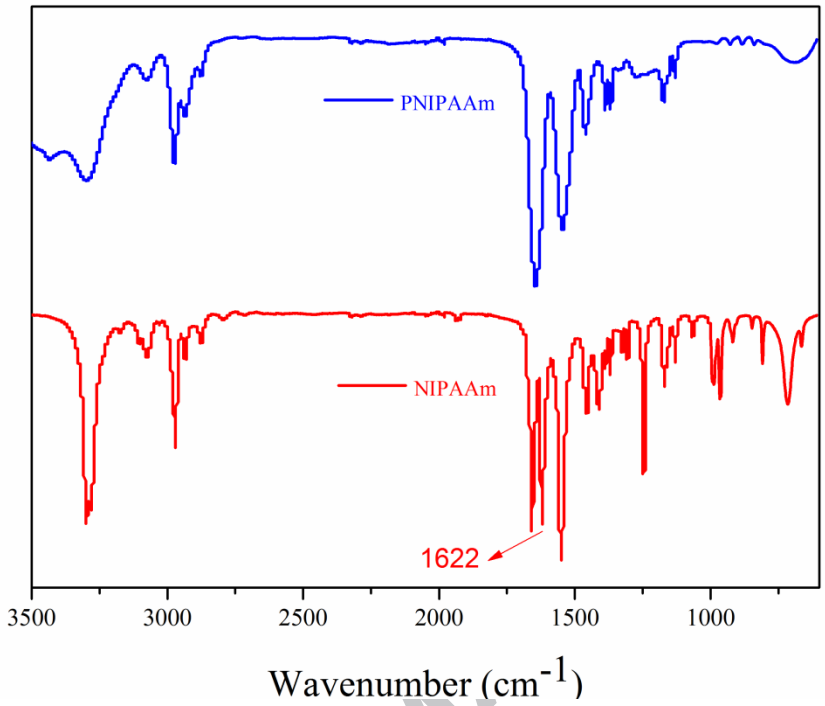

Fig. 2 FTIR spectra of PNIPAAm and NIPAAm

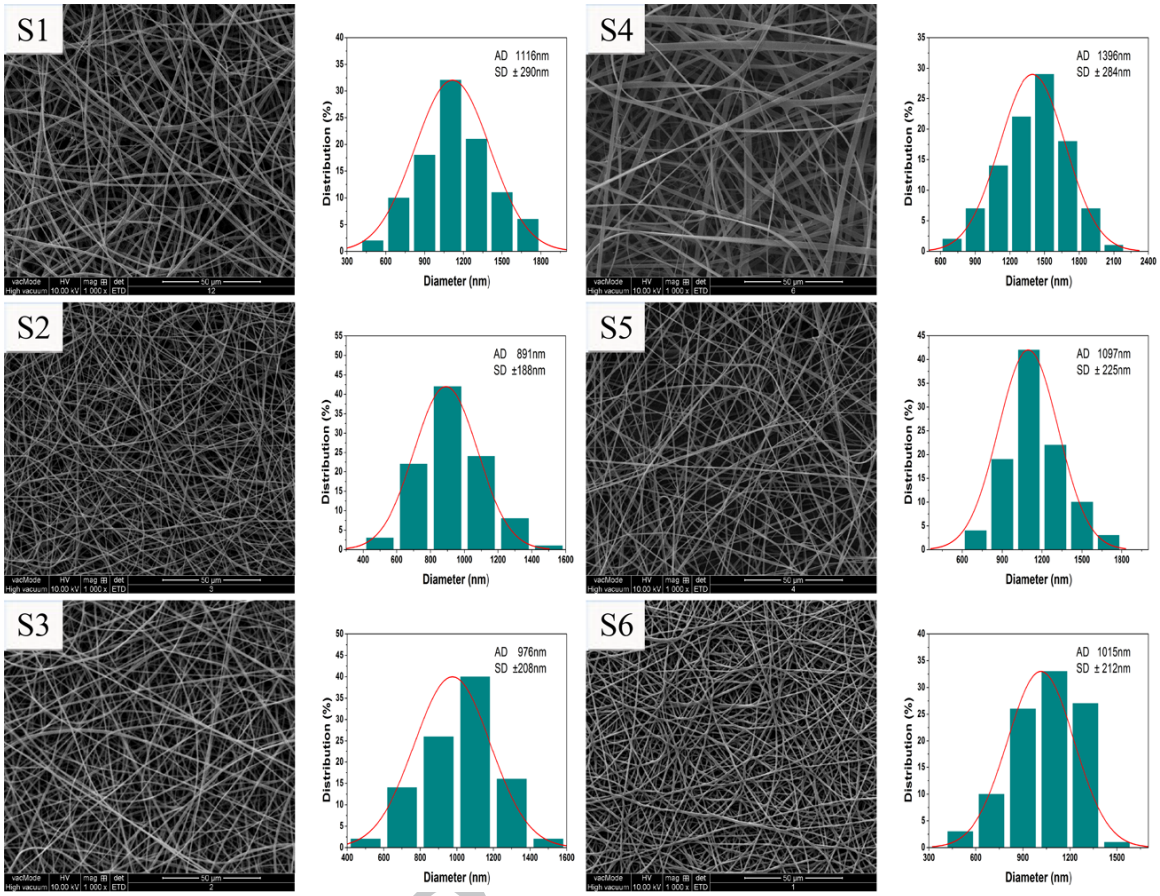

Fig. 3 SEM images and diameter distributions of the fibers. The scale bar in the SEM images represents 50 μm.

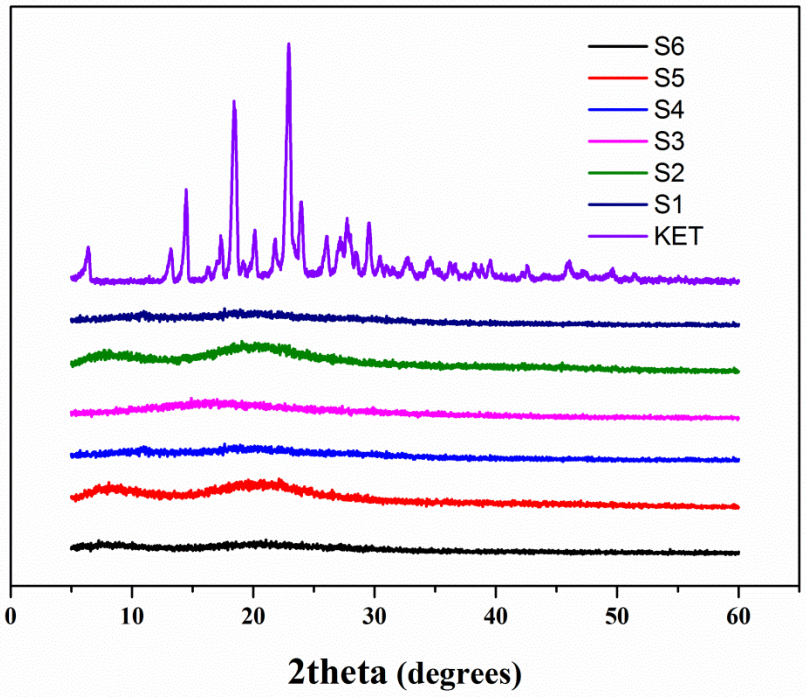

Fig. 4 XRD patterns of the fibers and pure KET.

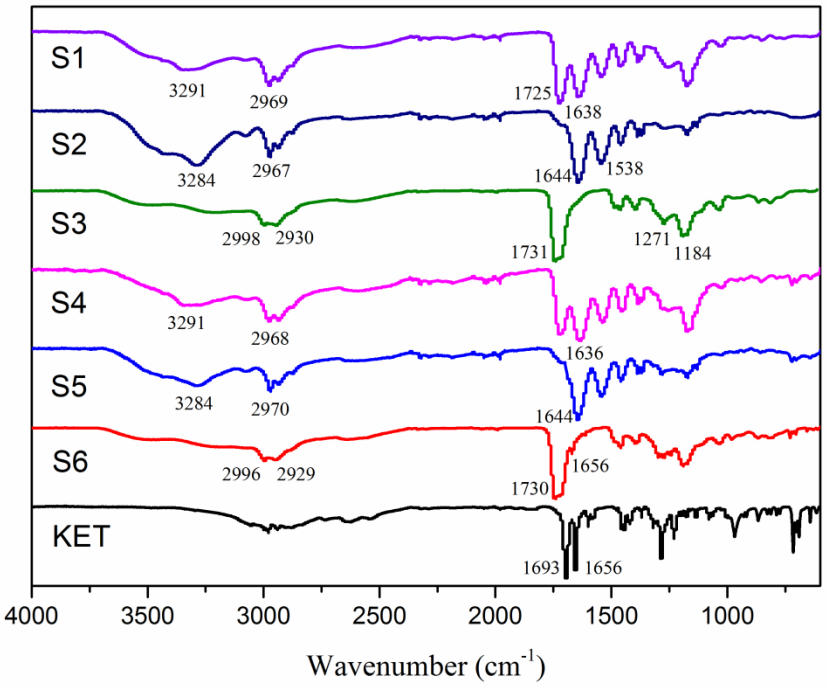

Fig. 5 FTIR spectra of the fibers and pure KET.

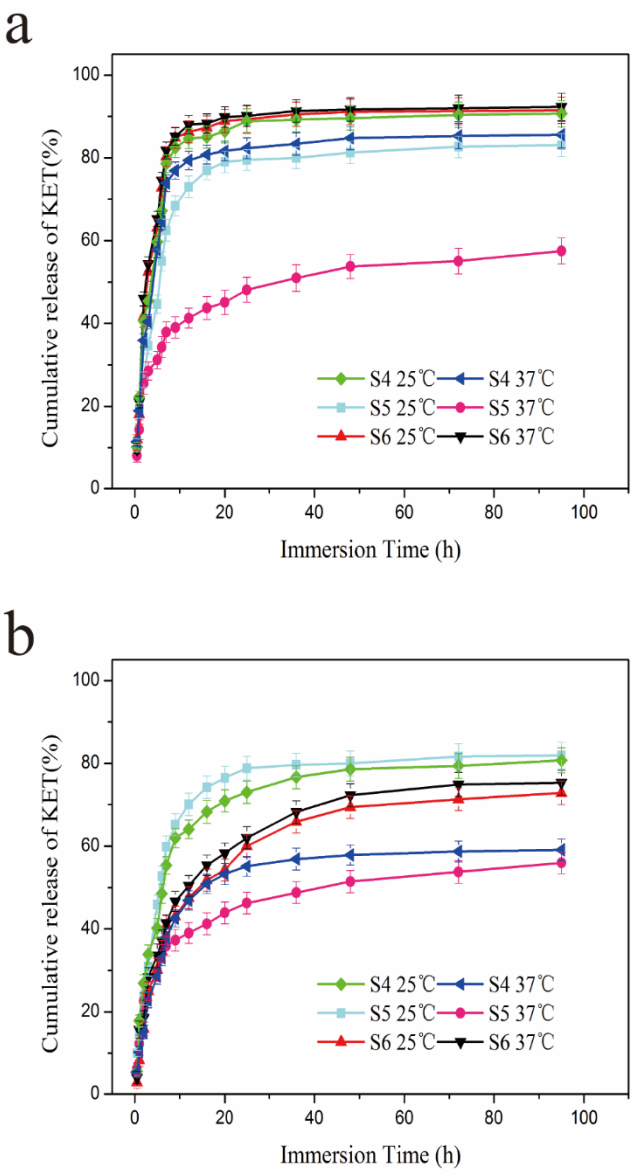

Fig. 6 The *in vitro* release profiles of KET from the drug-loaded fibers in (a) PBS (pH7.4) and (b) acetic acid buffer (pH4.5). Data are reported as mean ± S.D. from three independent experiments.

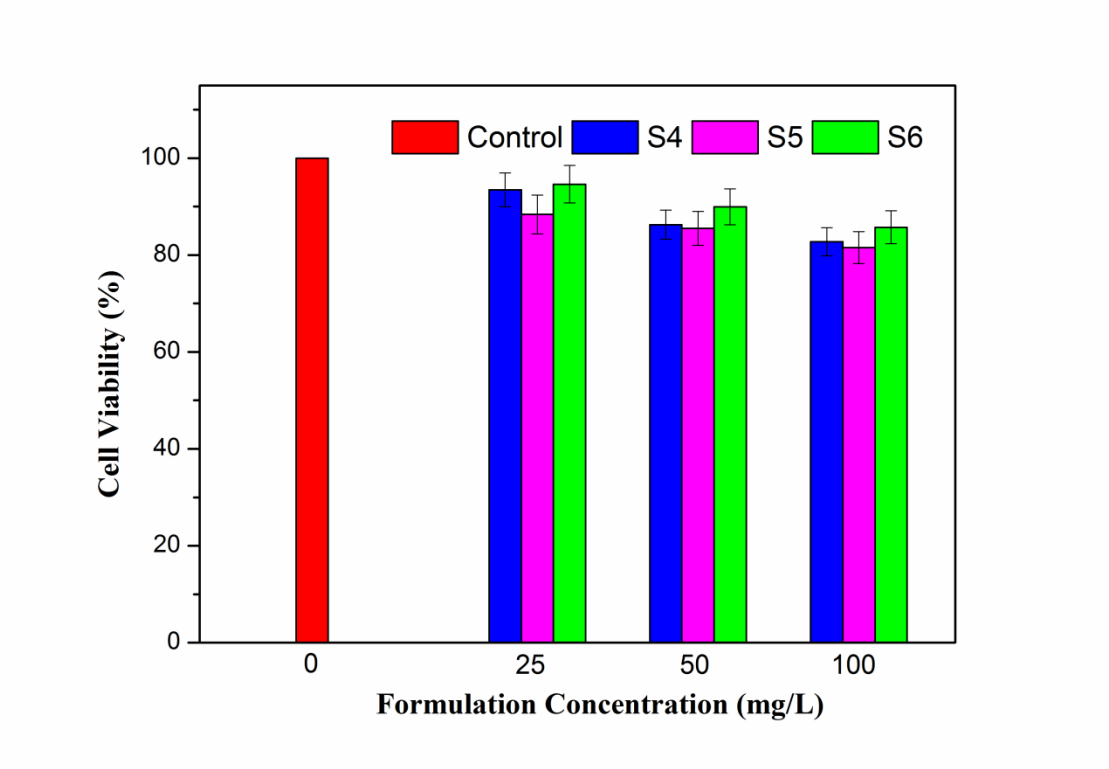

Figure 7. MTT assay results of all the formulations. Data are reported as mean ± S.D. from three independent experiments. 18wells for each condition in each experiment were used to test in each independent experiment.

Tables

Dual-responsive drug delivery systems prepared by blend electrospinning

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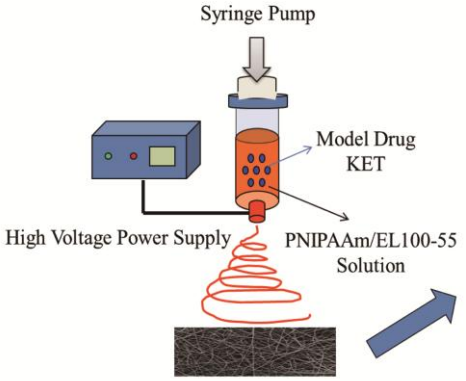
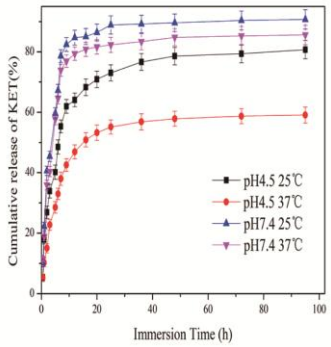
Table 1. Details of the electrospinning solutions prepared in this work.

Sample	Solution contents	PNIPAAm to EL100-55 ratio (w/w)	KET concentration (% w/v)
S1	PNIPAAm/EL100-55	1:1	---
S2	PNIPAAm	---	---
S3	EL100-55	---	---
S4	PNIPAAm/EL100-55	1:1	4.0
S5	PNIPAAm	---	4.0
S6	EL100-55	---	4.0



Drug-loaded Nanofibers



Drug Release Behaviors