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Dual temperature and pH responsive nanofiber formulations prepared by electrospinning

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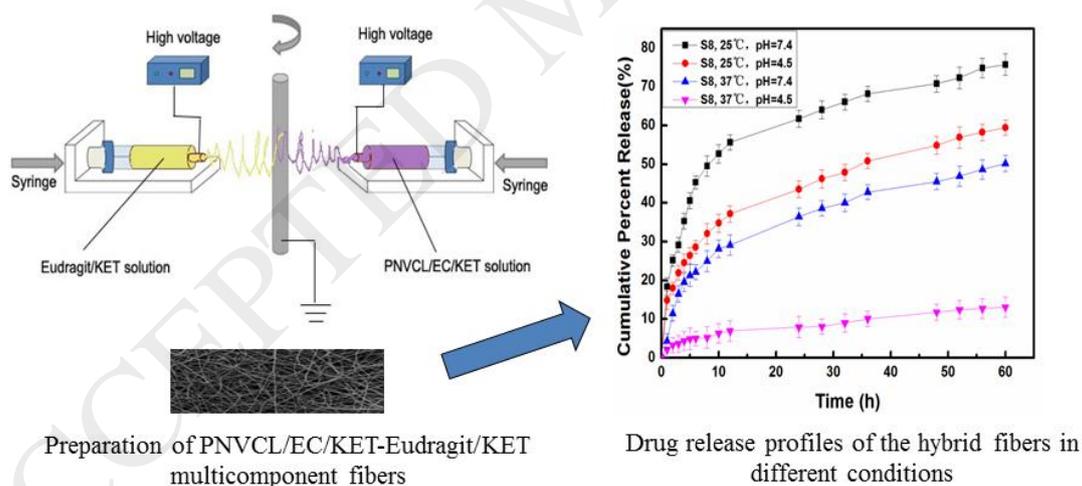
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Graphical Abstract



Highlights

- Thermosensitive PNVCLEK was synthesized by radical polymerization.

- PNVCL/EC/Eudragit hybrid fibers were fabricated by twin-jet electrospinning.
- The wettability of PNVCL-containing fiber changed as the temperature increased.
- KET loaded-fibers showed dual-sensitive properties with sustained release.

Abstract

We report a dual-responsive drug delivery system prepared by electrospinning. Blend fibers of poly(N-vinylcaprolactam) (PNVCL) and ethyl cellulose (EC) were first prepared, with the aim of developing thermoresponsive sustained release formulations. Eudragit L100-based fibers were then generated to yield pH-sensitive materials. Attempts to produce three-polymer fibers of EC, PNVCL and Eudragit were unsuccessful, and therefore hybrid mats containing two fiber populations (one made of PNVCL/EC, one comprising Eudragit) were instead fabricated by twin-jet electrospinning. Analogous drug-loaded versions of all the formulations were also prepared containing ketoprofen (KET). The fibers were largely smooth and homogeneous, and the addition of KET did not affect their morphology. The PNVCL-containing fiber mats changed from being hydrophilic to hydrophobic when the temperature was increased through the lower critical solution temperature of 33 °C. *In vitro* drug release profiles showed that the hybrid fiber mats were able to combine the properties of the three polymers, exhibiting both pH-sensitive and thermosensitive properties with sustained release. In addition, they were found to be nontoxic and suitable for cell growth. This study therefore demonstrates that PNVCL/EC/KET-Eudragit/KET multicomponent fiber mats comprise effective and biocompatible materials for targeted drug delivery.

Keywords: electrospinning, pH-sensitive, thermosensitive, drug delivery, Eudragit,

poly(N-vinylcaprolactam)

Introduction

Electrospinning is a widely-explored method to fabricate non-woven fiber mats. In the most commonly used solution spinning approach, electrical energy is employed to convert a polymer solution in a volatile solvent into one-dimensional fibers with diameters on the micro- or nanoscale. The resultant mats have large surface area-to-volume ratios and high porosity. These properties permit electrospun fibers to be used as tissue engineering scaffolds and drug delivery systems [1-3], in addition to a range of other applications. The simplest fibers are made of a single polymer, often with a functional component such as a drug embedded. However, it can be desirable to blend different polymers into fibers or prepare mats containing two different types of polymer fiber. This can impart the overall composite with enhanced properties, for instance in terms of mechanical strength or wettability [4-6].

Although producing scaffolds containing a mixture of polymers can be advantageous, it can also be challenging. To prepare monolithic materials where each fiber contains a blend of polymers requires both to be dissolved in the same solution, and a particular combination of polymers in a single solution may not be electrospinnable. Alternatively, it may be that the polymers of interest cannot be dissolved in the same solvent. To resolve this issue, different types of spinning can be implemented, including coaxial, side-by-side, tri-axial and other complex electrospinning processes [7-10]. However, these all involve dispensing multiple liquids through a single spinneret, and interfacial interactions between the different working fluids make these multiple-fluid processes very difficult to implement. An alternative and simpler approach is to simultaneously dispense multiple fluids onto a single collector from separate spinnerets. The product of this is a fiber mat with two or more different populations of fibers. The separate dispensing of the working fluids obviates any problems of interactions arising between the fluids being dispensed, and

may provide new routes for developing novel multi-functional materials [11, 12]. To this end, Ding et al. designed a multi-jet electrospinning device, which can be used to generate uniform mats containing two different types of fibers [13]. Because the two polymer solutions are completely separate, Ding's approach does not require the polymers of interest to be soluble in the same solvent. Despite this, the multi-jet approach has not received much attention in the literature to date.

Electrospun fibers have attracted particular attention as drug delivery systems (DDSs) over the last decade or so [14-16]. An effective DDS should be able to release its drug cargo at a predetermined rate in the desired location [17]. One route by which this might be achieved is to use stimuli-responsive materials [18-20]. These undergo a change in their physicochemical properties in response to variation in external conditions such as pH, temperature, or the presence of certain enzymes [21].

Thermosensitive drug delivery systems are well known in the literature, and are commonly based on polymers such as poly(*N*-isopropylacrylamide) (PNIPAM) or poly(*N*-vinylcaprolactam) (PNVCL) [15, 22, 23]. These materials undergo distinct hydrophilic/hydrophobic phase transitions at a particular temperature. For instance, PNVCL has a lower critical solution temperature (LCST) of 33 °C, being hydrophilic and water soluble below this temperature and hydrophobic above it [24]. The most commonly explored thermosensitive polymer is PNIPAM [25, 26], but PNVCL has advantages of reduced cytotoxicity after hydrolysis: PNIPAM produces toxic small-molecule amide compounds after degradation [27, 28].

A range of thermoresponsive DDSs based on PNIPAM and PNVCL have been successfully prepared by electrospinning. For example, PNIPAM/poly(ethylene oxide) (PNIPAM/PEO) nanofibers loaded with vitamin B were fabricated by blend electrospinning, and the drug release rates could be controlled by adjusting the temperature of the release medium, the weight ratio of PNIPAM/PEO, and the drug loading [29]. In other work, Liu et al. prepared fibers from the thermo-sensitive copolymer poly(*N*-vinylcaprolactam-*co*-methacrylic acid) and found that the drug release profiles were greatly influenced by the environmental temperature [30].

In addition to temperature, pH responsiveness can be a potent way to control

release from a DDS. This allows scientists to exploit the changes in pH that arise as a formulation taken orally passes through the digestive tract, for instance. A range of pH sensitive polymers exists. One is Eudragit L100, which is widely used in the pharmaceutical industry (e.g. as enteric coatings for tablets). Eudragit L100 is synthesized from methacrylic acid and methacrylic acid methyl ester, and dissolves only at pH values higher than 6.0. When the pH value is lower than 6.0, the polymer is insoluble in aqueous media [31]. This makes it potent for delayed release formulations and the targeting of drug release to the lower reaches of the intestinal tract [32].

The vast majority of work reported to date deals with DDSs able to respond to a single stimulus. There are only a limited number of studies concerning dual-responsive systems able to react to both temperature and pH [33-36]. Further, most of these were produced by generating new copolymers and processing them into hydrogels and micelles. This is problematic because the synthesis of new copolymers is generally complicated, high-cost, and time consuming. Here, we adopt a straightforward, effective, and low-cost twin-jet electrospinning process to produce dual-responsive drug delivery systems from well-known and easily obtainable polymers. The resultant formulations have a number of potential benefits over micellar formulations (for instance, an electrospun fiber mat has morphology which closely resembles the extracellular matrix). This work provides a simple route to prepare dual- or multi responsive DDSs, yielding functional formulations and additionally offering new knowledge on multi-jet electrospinning.

Ketoprofen (KET), a non-steroidal anti-inflammatory drug was used as the model drug in this work. Materials were prepared using PNVCL, ethyl cellulose (EC, a water-insoluble and non-toxic polymer) [37, 38], Eudragit L100, and mixtures of these. PNVCL and EC could be mixed in a co-dissolving solution, and the latter then used to prepare blend fibers, with the aim of improving the spinnability of the system and producing thermoresponsive materials with sustained release behaviors. When attempts were made to dissolve the three polymers (PNVCL, Eudragit, and EC) in a single solution to produce fibers able to respond to both pH and temperature stimuli,

precipitation was observed. Therefore, PNVCL/EC-Eudragit mats comprising two different populations of fibers were prepared using the dual source approach (**Figure 1**), with separate solutions of Eudragit/KET and PNVCL/EC/KET. Morphological studies, physical form characterization, *in vitro* drug release, and biocompatibility assays on the various fibers were undertaken to determine their properties and functional performance.

Experimental

Materials

N-vinylcaprolactam (NVCL) was purchased from Sigma-Aldrich Ltd. (China), while Eudragit L100 (average molecular weight ca. 135,000) was provided by Rohm GmbH (Germany). Azobisisobutyronitrile (AIBN), anhydrous ethanol, dimethylformamide, methanol and absolute ether were obtained from the Sinopharm Chemical Reagent Co., Ltd (China). Ethyl cellulose (EC, 6-9 m Pa·s) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, 99.5%) were acquired from the Aladdin Chemistry Co., Ltd. (China). Ketoprofen (KET) was purchased from Beijing J&K Scientific Co., Ltd. (China). Phosphate-buffered saline (PBS), penicillin, trypsin, and thiazolyl blue (MTT) were sourced from Sigma-Aldrich (USA). L929 cells were provided by the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, China). Dimethyl sulfoxide (DMSO) and DMEM culture medium were obtained from Jinuo Biological Medicine Technology Ltd. (China). All water used was doubly distilled before use.

PNVCL synthesis

PNVCL was synthesized by radical polymerization using AIBN as an initiator [39]. 1.0g NVCL and 0.001g AIBN were dissolved in DMF (1 mL). Dry nitrogen was bubbled through the solution for 20 min prior to polymerization, which was carried out at 70 °C for 8 h under a nitrogen atmosphere. The product was dissolved in 1 mL of methanol, and precipitated with an excess amount of diethyl ether before being dried under vacuum. Next, the polymer was dissolved in 10 mL of deionized water

and dialyzed against distilled water for 48 h, using dialysis tubing with a molecular weight cut-off of 3.5 kDa. This was followed by freeze drying. Successful polymerization was evidenced by ^1H nuclear magnetic resonance in D_2O (AV-400 instrument, Bruker, Germany). Molecular weights (M_w , M_n) and molecular weight distributions were quantified by gel permeation chromatography (GPC) measurements. These were undertaken 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 °C. Calibration was performed with standard polystyrene samples.

Preparation of electrospinning solutions

EC and PNVCL were dissolved in ethanol at room temperature, with magnetic stirring performed overnight to ensure complete dissolution. The component ratios of EC to PNVCL were 2:1 (w/w) in the blend fibers, and in all cases the total concentration of polymer was 25 % (w/v). Solutions of Eudragit L100 were also prepared in ethanol at 25 % (w/v). KET was added into selected solutions at a drug to polymer ratio of 1:5 (w/w). Full details of the solutions prepared are presented in **Table 1**.

Electrospinning

Solutions were loaded into 5.0 mL plastic syringes fitted with a stainless steel needle (internal diameter 0.5 mm). The syringe was mounted on a syringe pump (KDS100, Cole-Parmer, USA). Liquid was expelled at a flow rate set to 0.8 mL/h, and fibers fabricated under an applied voltage of 14 kV (ZGF-2000 power supply, Shanghai Sute Electrical Co. Ltd., China). Experiments were performed at ca. 40 % relative humidity, and at a temperature of approximately 25 °C. The grounded collector (a flat piece of aluminum foil of 10 × 10 cm in size) was placed 15 cm from the needle tip. The hybrid mats (S7 and S8 in **Table 1**) were prepared by dual-source electrospinning, as shown in **Figure 1**. This uses two independent syringe pumps (both KDS100 models) and two ZGF-2000 power supplies. Liquid was dispensed from the two pumps

simultaneously to produce a product comprising two populations of fibers. The voltage, flow rate, spinneret-to-collector distances and environmental parameters were identical for both sources, and the same as those detailed above. However, rather than a flat piece of foil a rotating mandrel (with a diameter of 80 mm) was used to collect the fibers. The mandrel was grounded and rotated at 50 rpm. In all cases, electrospinning was performed for 6 hours, after which the fiber membranes obtained were stored in a vacuum oven to remove any residual solvent.

Fiber characterization

The fiber morphology was studied by scanning electron microscopy (SEM; JSM-5600 LV microscope, JEOL, Japan) at a voltage of 10 kV. Samples were cut from the fiber mats and sputtered with gold for 60 s, under argon. The mean diameter of the fibers in each sample was calculated using the Image J software (National Institutes of Health, USA) from measurements taken at more than 100 locations in SEM images.

X-ray diffraction (XRD) data were acquired on a D/Max-BR diffractometer (Rigaku, Japan). The instrument is supplied with Cu K α radiation (40 kV / 30 mA), and patterns were collected over the 2 θ range 5–60°. Fourier transform infrared (FTIR) spectroscopy was performed using a Nicolet-Nexus 670 FTIR spectrometer (Nicolet Instrument Corporation, USA). Spectra were obtained over the range 500–4000 cm⁻¹ at a resolution of 2 cm⁻¹.

The water contact angle of the fiber mats was determined on a contact angle analyzer (DSA 30, Krüss GmbH, Germany) in air. A drop of water (ca. 5 μ L) was placed onto the surface of the fibers and the contact angle recorded immediately. The temperature was varied from 20 to 45 °C with the aid of a heating platform (XMTD-204, JTHF Company, China) to explore how the contact angle varied upon heating. Five measurements were recorded for each sample, and the results are reported as mean \pm S.D.

In vitro drug release

Drug release experiments were carried out at two different temperatures and at two different pH values in a thermostatic shaking incubator (Jintan Instrument Co. Ltd., China), at a speed of 110 rpm. 50 mg of each sample was separately immersed in 30 mL of a release medium (pH 7.4 phosphate buffered saline [PBS] or pH 4.5 acetate buffer). At predetermined time points, 1 mL of the test medium was withdrawn and an equal amount of fresh buffer (pre-heated to either 25 or 37 °C) was added. KET release was quantified using a UV-visible spectrometer (UV-1800, SHJH Company, China) at a wavelength of 260 nm. The cumulative release was calculated using the equations:

$$M_n = C_n V + V_1 \sum_{n=1}^n C_{n-1}$$

$$W = M_n / M \times 100\%$$

Where M_n and C_n are the cumulative mass of drug released and the concentrations of KET at each time point, V and V_1 the volumes of the total release medium and the aliquots taken, M is the total mass of KET in the fibers, and W the cumulative release percentage. All release studies were performed in triplicate, and the results are given as mean \pm S.D.

Cell viability

To prepare samples for *in vitro* cellular toxicity tests, fibers were electrospun directly onto cover slips according to protocols in the literature [40]. The fiber-covered slips were put into 24-well plates, with untreated cover slips used as a negative control. The plates were sterilized by alcohol steam for 24 h. For cell culture, 400 μ L of dissociated L929 fibroblasts (1.0×10^4 cells/mL, in DMEM supplemented with 10 % v/v FBS and 1 % v/v penicillin–streptomycin solution) was added into each well and cultured in an incubator (37 °C, 5 % CO₂). After 1, 3, or 5 days' culture, the culture medium was

withdrawn and replaced with 360 μL of fresh DMEM and 40 μL of an MTT solution (5 mg/mL thiazolyl blue in PBS). Following incubation for an additional 4 h (37 $^{\circ}\text{C}$, 5 % CO_2), the medium was removed and 200 μL of DMSO added to each well, after which the plates were shaken for 20 min at 37 $^{\circ}\text{C}$. The resultant solution was transferred to 96-well plates and the number of cells assessed via the OD values at 570 nm. The latter were quantified on a microplate reader (Multiskan, ThermoFisher, USA). Three independent MTT assays were carried out, with 6 replicates per assay.

Results and discussion

Synthesis of PNVCL

^1H NMR spectra of NVCL and PNVCL are shown in **Figure S1**. The spectrum of NVCL (**Figure S1a**, D_2O , 400 MHz) has peaks at δ values of 7.02 (1H, 7-H), 4.75 (1H, 6b-H), 4.54 (H, 6a-H), 3.59 (2H, 5-H), 2.54 (2H, 4-H) and 1.62 (6H, 1,2,3-H). The spectrum of PNVCL (**Figure S1b**, D_2O , 400 MHz) has peaks at δ values of 4.21, 3.23, 2.35 and 1.71. It is clear that peaks of the ethylene group in NVCL have disappeared after polymerization. These results agree with a previous study [41], and verify the successful polymerization of NVCL. The molecular weights (M_w and M_n) and molecular weight distributions (PDI) of PNVCL determined by GPC were 99,170, 73,924, and 1.15, respectively.

Fiber morphology

PNVCL, EC and Eudragit can all be successfully electrospun alone, and it also proved possible to prepare blend fibers of EC and PNVCL, with the goal of generating thermoresponsive extended release formulations. We further attempted to prepare dual-responsive fibers containing EC, PNVCL and Eudragit. However, precipitation was observed when making a solution containing all three polymers in a single solution (1:1:1 w/w ratio, total polymer concentration 25% w/v) (see Supporting Information, **Figure S2**). It is not completely clear why this precipitation arises, but it

is believed to be a result of favorable interactions between the polymers causing them to aggregate into particles when all three polymers are combined. Therefore, the dual source spinning method was applied in order to obtain composite EC/PNVCL/Eudragit fiber mats with both thermo- and pH-sensitivity.

SEM images of all the fibers prepared are given in **Figure 2**. In all cases, fibers have been successfully fabricated. S1 (EC), S2 (PNVCL), S3 (Eudragit) and S4 (Eudragit/KET) comprise smooth and homogeneous nanofibers with average diameters ranging from 300 – 500 nm. This is as expected: the polymers EC [37], PNVCL [42] and Eudragit [15] have been shown to have good spinnability and to form high-quality drug-loaded fibers. In contrast, the S5 (PNVCL/EC; 537 ± 220 nm), S6 (PNVCL/EC/KET; 668 ± 352 nm), S7 (PNVCL/EC-Eudragit; 586 ± 287 nm) and S8 (PNVCL/EC/KET-Eudragit/KET; 578 ± 270 nm) fibers are less uniform in diameter, presumably due to the inclusion of both EC and PNVCL. Although the hybrid fiber mats S7 and S8 contain two populations of fibers with different compositions, in terms of their morphologies these cannot be distinguished (see **Figure 2**). Comparing the fibers with and without KET (for instance, S3 and S4; S7 and S8), their diameters and morphologies are very similar. The addition of KET thus appears to have little influence on the fibers.

X-ray diffraction

XRD patterns of all the fibers (S1-S8) and pure KET are presented in **Figure 3(a)**. KET displays a number of characteristic reflections at 6.4° , 13.0° , 14.2° , 18.2° , 20.3° , 21.6° , 22.9° , 23.8° , 26.3° and 29.6° 2θ . This confirms the raw drug to be a crystalline material, consistent with the literature [43]. In contrast, in the patterns of the fibers there are no distinct Bragg reflections visible. This demonstrates them to be amorphous materials. In the case of the drug-loaded fibers (S4, S6 and S8), the reflections of KET have disappeared, and therefore the fibers comprise amorphous solid dispersions. Similar results have been reported in many previous studies in the

literature [44-46]. Solvent evaporation during electrospinning is very rapid, and therefore there is insufficient time for the drug molecules to order themselves into a regular crystalline arrangement during the solidification process [47, 48]. The random arrangement of molecules in the solution phase is carried through into the solid state, leading to the drug molecules forming a solid solution in the polymer matrix.

FTIR spectroscopy

FTIR spectra of all the fiber samples and KET are given in **Figure 3(b)**. S1 (EC alone) shows characteristic peaks arising from the OH group (3474 cm^{-1}) and C-O-C stretches (1107 cm^{-1}). The spectrum of PNVCL (S2) exhibits a characteristic absorption at 1635 cm^{-1} (C=O stretching). The Eudragit fibers (S3) show a peak at 1178 cm^{-1} , which corresponds to ester (C-O-C) stretching bands, and a strong absorption at 1728 cm^{-1} caused by the stretching of the carbonyl groups. In addition, all three polymers display peaks in the region of $2750\text{-}3000\text{ cm}^{-1}$ (CH₃, CH₂, and CH stretches) [49].

For the composite fibers S5 and S6, both the characteristic peaks from EC (C-O-C stretches) and PNVCL (C=O stretching) can be observed, indicating the successful incorporation of the two polymers in the fibers. Similar, the presence of peaks from the multiple components in the S7 and S8 fiber mats indicate the existence of EC, PNVCL and Eudragit in the composite fibers.

The FTIR spectrum of KET displays characteristic peaks at 1695 cm^{-1} and 1645 cm^{-1} , representing the stretching vibration of the COOH and ketone groups, respectively. The peak at 1645 cm^{-1} can be seen in the drug-loaded Eudragit fibers (S4). Similarly, the peaks of S6 at 1640 cm^{-1} and S8 (1638 cm^{-1}) are enhanced in intensity compared with the drug free S5 and S7. These observations demonstrate the successful combination of KET with the polymers. However, the peak at 1695 cm^{-1} of KET cannot be observed in the drug-loaded fibers S4, S6 and S8. In the case of S4 and S8 there are two potential explanations: this peak may be obscured by the Eudragit vibration at 1728 cm^{-1} , or the absence could be explained by the phase

transformation of KET during the electrospinning process. In its crystalline form, KET molecules are bound together in dimers through intermolecular hydrogen bonds, resulting in the appearance of the distinct peak at 1695 cm^{-1} . Therefore, the absence of this band in the KET-loaded samples indicates a lack of dimers, agreeing with the results from XRD.

Contact angles

The contact angles for all the fibers are depicted in **Figure 4**. The pure EC fibers (S1) are hydrophobic (contact angles $> 130^\circ$) regardless of the temperature, with only a very small increase in contact angle seen across the temperature range studied. The contact angles of the Eudragit-based fibers (S3 and S4) were around 0° , and again remain constant with temperature. This arises because these materials can be dissolved by the addition of water, resulting in very low contact angles. In contrast to EC and Eudragit, as the temperature is increased from 25 to 45 °C the PNVCL-containing materials change abruptly from being hydrophilic to hydrophobic. This phenomenon occurs when the temperature increases through the LCST of 32-34 °C.

When the temperature is below the LCST (33 °C), the hydrophilic C=O and N-H groups in the PNVCL chains interact significantly with water molecules to form intermolecular hydrogen bonds. Consequently, the fibers exhibit hydrophilic properties. When the temperature rises above the LCST, the formation of intramolecular hydrogen bonds between the C=O and N-H groups of PNVCL is favored over interactions with water, leading to a collapsed globular conformation of the polymer chains. As a result, the fibers are hydrophobic above the LCST [50].

Drug release

The drug release profiles of the Eudragit/KET nanofibers (S4) at different pH and temperatures are given in **Figure S3(a)**. Since Eudragit is pH-sensitive, the S4 fibers have pH-dependent drug release behavior regardless of the temperature. The fibers

have a more rapid drug release rate in PBS than in an acidic solution. At pH=7.4, KET release reaches ca. 90% in 24 h. However, at pH 4.5 drug release reached only around 48% after 60 h. Eudragit L100 is only soluble above pH 6.0, where sufficient COOH groups in the polymer chain become ionized [51]: thus, at pH 7.4 drug release is likely to be controlled by the rate of polymer dissolution, which is fairly rapid. In contrast, at pH 4.5 the polymer is insoluble, and the KET molecules must diffuse through it to reach the release medium. This is a slower process than polymer dissolution.

Figure S3(b) depicts the release of KET from the PNVCL/EC/KET nanofibers (S6) at different pH and temperatures. The fibers showed similar release behaviors at the two pH values. However, they displayed promising temperature-dependent drug release profiles. At 25 °C, the amount of KET released from the fibers is greater than at 37 °C. This can be explained by the different surface wettability of PNVCL at the two temperatures. A hydrophilic carrier tends to give faster release than a hydrophobic analogue [52]: when the temperature is 37 °C PNVCL shows very hydrophobic properties, while it is hydrophilic at 25 °C.

In vitro drug release profiles for the hybrid fiber mats (S8) are given in **Figure 5**. The S8 fiber mats combine the pH-sensitivity of Eudragit and thermosensitivity of PNVCL, and thus display dual-responsive drug release properties. A greater extent of release is observed at pH 7.4 than at 4.5, and similarly at 25 °C (below the LCST of PNVCL) more drug was freed from S8 than at 37 °C. At 37 °C and pH 4.5, the lowest extent of KET release is observed, just 15% after 60 h. In contrast, S8 displayed the greatest amount of release and most rapid rate at 25 °C and pH 7.4. After 60 h, more than 75 % of the KET loading has been freed into solution.

The mechanism of release from S8 was modelled with the Peppas equation [53]:

$$Q = kt^n$$

where Q is the drug release percentage, t is the release time, k is a rate constant, and n is an exponent that indicates the drug release mechanism. The results of this fitting are shown in **Table 2**, and the fits are shown in the Supporting Information (**Figures S4 –**

S7). These exponents are smaller than 0.45, suggesting that typical Fickian diffusion mechanisms are dominant [54].

Biocompatibility

The results of MTT cell viability measurements for L929 cells exposed to the fibers are given in **Figure 6**. In all cases, the cell numbers increased from day 1 to 5, indicating all the formulations to have high biocompatibility. The pure EC fibers (S1) led to the highest cell viability, which is accordance with the literature [15]. In contrast, cells cultured with pure PNVCL (S2) and Eudragit (S3) fibers did not grow as well. In the case of the S2 fibers, the morphology of the fiber samples could be expected to change during culture, since the experiments took place above the LCST of the polymer. The lower viabilities observed with the S3 fibers may be caused by the rapid dissolution of the Eudragit fibers in PBS. The composite fiber mats perform a little worse than pure EC fibers, but better than pure PNVCL and Eudragit fibers. Overall, these results demonstrate that the hybrid fibers are non-toxic to cells, which is very promising for potential biomedical applications. The KET-loaded Eudragit fibers (S4) showed virtually identical viabilities to the equivalent drug-free fibers (S3). Similar observations are also made for the KET-loaded composite fiber mats (S6 and S8) compared to their drug-free analogues (S5 and S7). The addition of KET therefore does not affect the biocompatibility of the nanofibers.

Conclusions

Nanoscale fibers made of EC, PNVCL, Eudragit and blends of these polymers are reported in this work. While it was possible to prepare blend thermosensitive sustained release fibers of EC and PNVCL using a standard single-needle electrospinning approach, to prepare dual-responsive fiber mats containing all three polymers it was necessary to use a two-source experiment and prepare hybrid mats with two fiber populations (EC/PNVCL and Eudragit) to avoid precipitation of the polymer solutions. Ketoprofen (KET) was chosen as a model drug, and analogous

drug-loaded fiber mats also fabricated. The fibers have regular and smooth morphologies in general, although the use of multiple polymers together causes greater inhomogeneity in the fiber populations. IR spectroscopy proved that the drug was successfully loaded into the nanofibers, and XRD data demonstrated that KET exists in the amorphous physical form in the drug-loaded fibers. The water contact angle of the PNVCL/EC blend nanofibers changes abruptly when the temperature is increased through the LCST of 33 °C. The pH-sensitive properties of the Eudragit-based fibers and thermoresponsiveness of the PNVCL/EC systems could be combined to give dual-responsive drug delivery systems by using the dual source technology to prepare hybrid mats containing both fiber types. For the latter, *in vitro* drug release tests determined that KET release at 25 °C is much faster than at 37 °C, while at pH 7.4 release is more rapid than at pH 4.5. The hybrid PNVCL/EC/KET-Eudragit/KET fiber mats also proved to be nontoxic and to allow cell proliferation. Overall, this study demonstrates that dual-responsive drug carriers for sustained release can be prepared by blending suitable fiber populations into a single mat.

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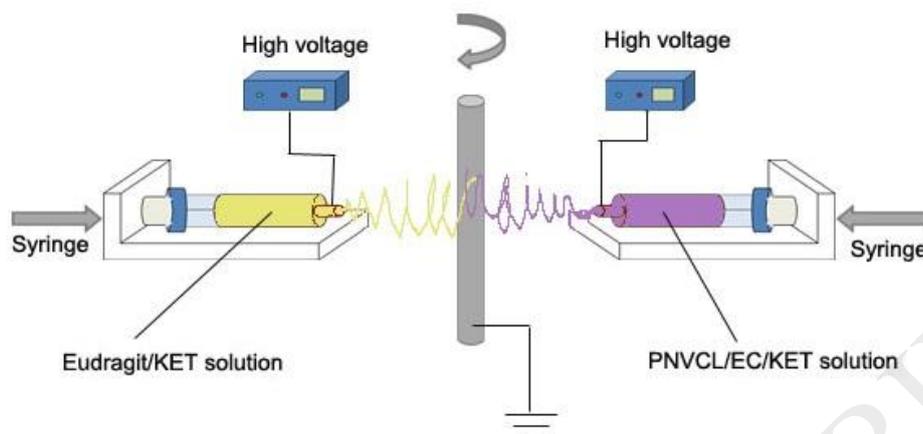


Figure 1. A diagram illustrating the dual-source and dual-power electrospinning device used to prepare hybrid mats.

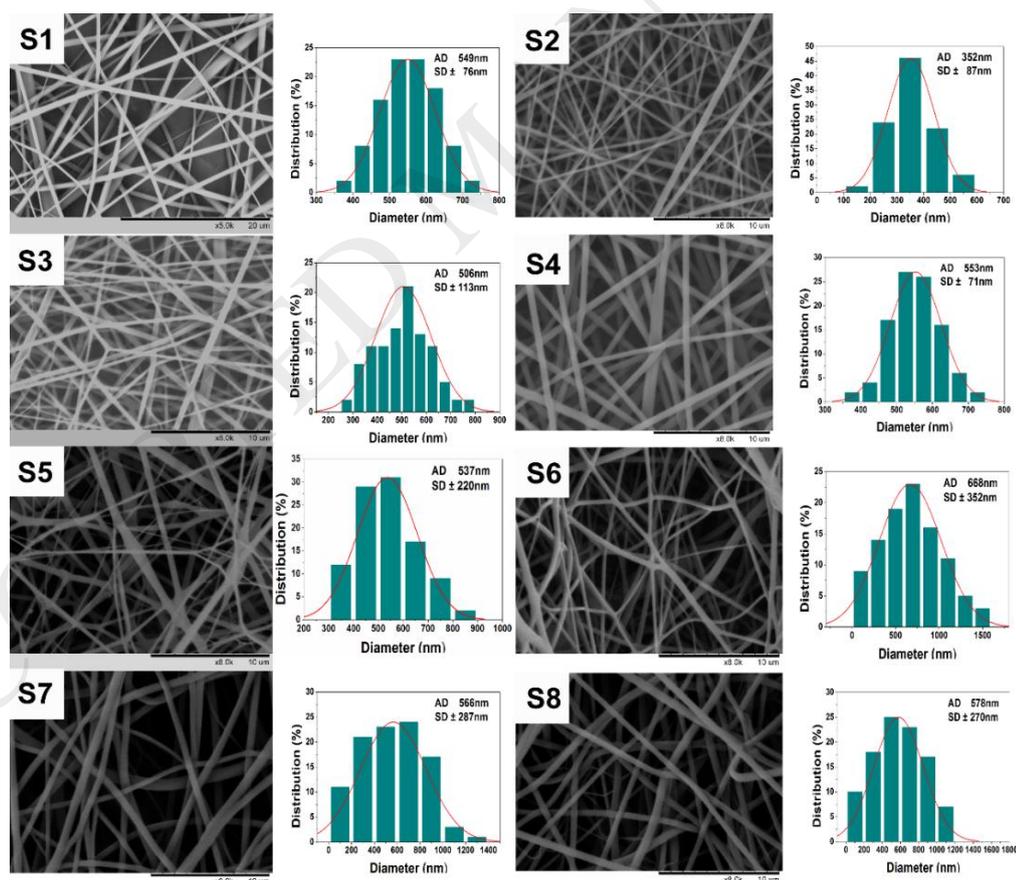


Figure 2. SEM images and diameter distributions of the fibers. Fiber compositions are as follows: S1: EC only; S2: PNVCL only; S3: Eudragit only; S4: Eudragit/KET; S5: PNVCL/EC; S6: PNVCL/EC/KET; S7:

PNVCL/EC-Eudragit; S8 PNVCL/EC/KET-Eudragit/KET. The scale bar represents 10 μm .

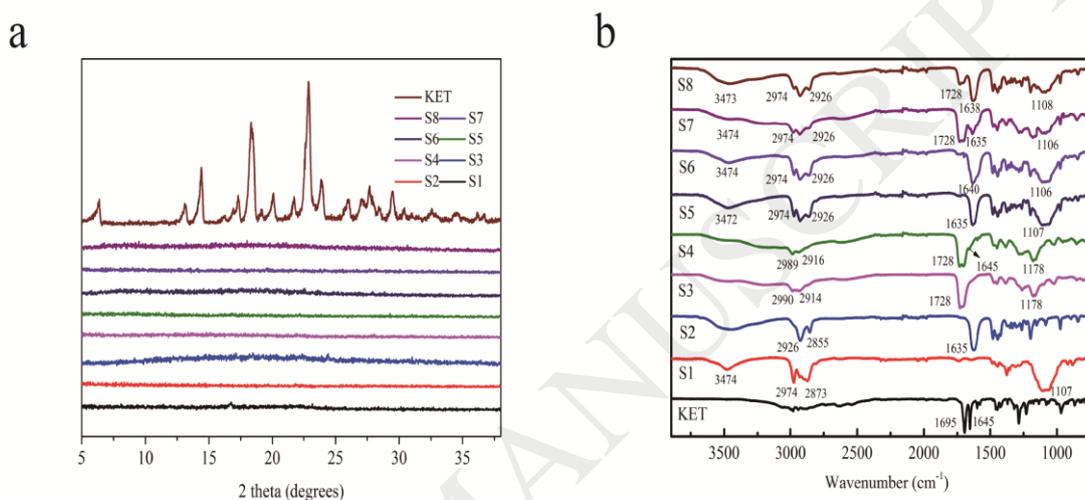


Figure 3. a) XRD patterns and b) FTIR spectra of the fibers and KET. Fiber compositions are as follows: S1: EC only; S2: PNVCL only; S3: Eudragit only; S4: Eudragit/KET; S5: PNVCL/EC; S6 PNVCL/EC/KET; S7: PNVCL/EC-Eudragit; S8 PNVCL/EC/KET-Eudragit/KET.

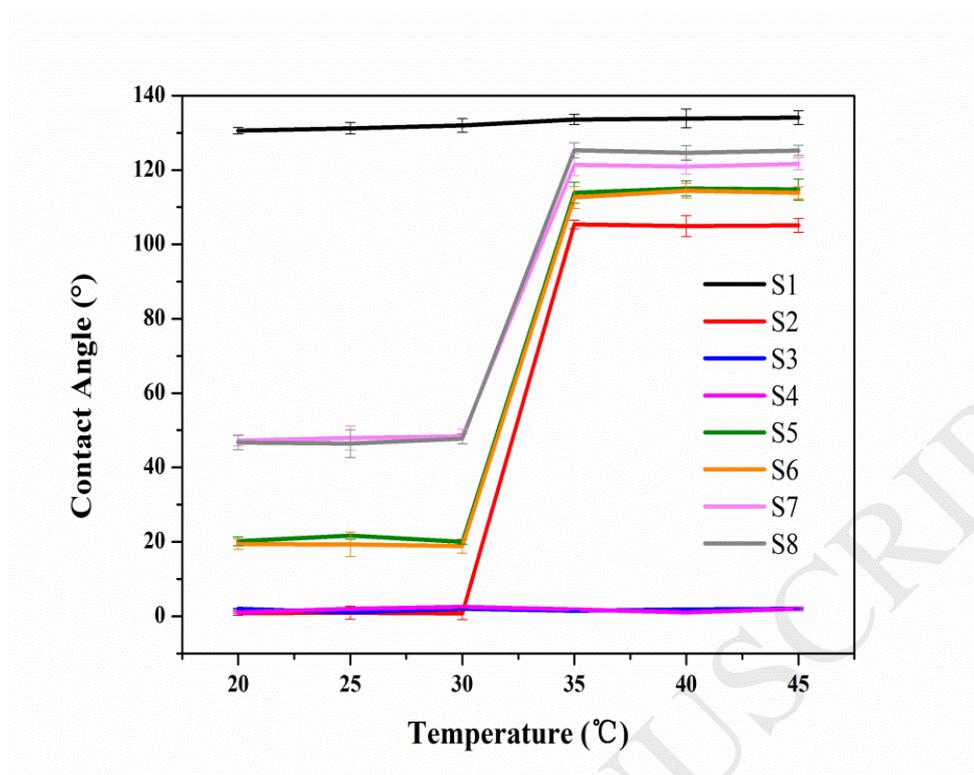


Figure 4. Contact angles of the fibers as a function of temperature. Fiber compositions are as follows: S1: EC only; S2: PNVL only; S3: Eudragit only; S4: Eudragit/KET; S5: PNVL/EC; S6 PNVL/EC/KET; S7: PNVL/EC-Eudragit; S8 PNVL/EC/KET-Eudragit/KET.

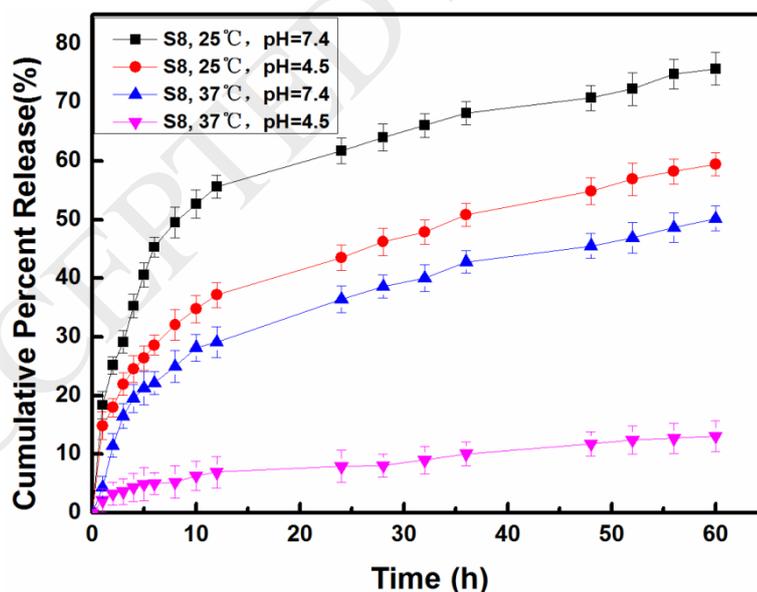


Figure 5. *In vitro* KET release profiles from the hybrid electrospun fiber mats of Eudragit and PNVL/EC (S8). Data are presented as mean \pm S.D. from three independent experiments.

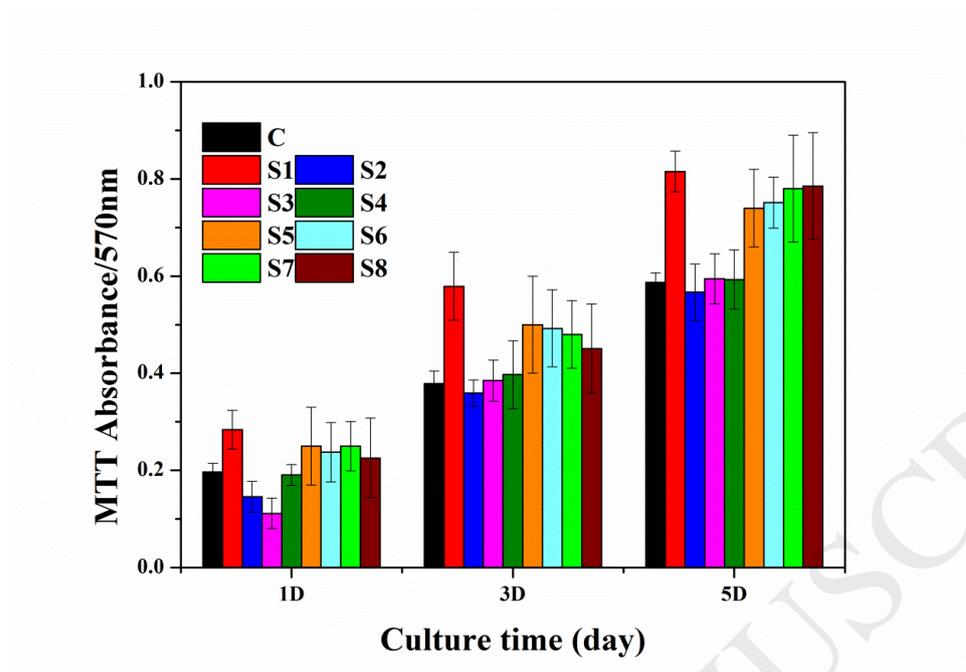


Figure 6 The viability of L929 cells grown on blank coverslips (C) and those coated with the various fiber mats. Fiber compositions are as follows: S1: EC only; S2: PNVCL only; S3: Eudragit only; S4: Eudragit/KET; S5: PNVCL/EC; S6 PNVCL/EC/KET; S7: PNVCL/EC-Eudragit; S8 PNVCL/EC/KET-Eudragit/KET.

Table 1. Details of the spinning solutions used in this work.

Sample	Solution components	Drug concentration (%)
S1	EC	---
S2	PNVCL	---
S3	Eudragit	---
S4	Eudragit /KET	5.0
S5	PNVCL/EC	---
S6	PNVCL/EC/KET	5.0
S7	1. PNVCL/EC 2. Eudragit	---
S8	1. PNVCL/ EC/KET 2. Eudragit/KET	5.0

Table 2. The results of fitting the Peppas equation to release from S8.

Conditions	25 °C	37 °C
pH 7.4	$Q = 25.237t^{0.275}$ ($R^2=0.9580$)	$Q = 10.913 t^{0.375}$ ($R^2=0.9784$)
pH 4.5	$Q = 15.743t^{0.325}$ ($R^2=0.9964$)	$Q = 2.272t^{0.420}$ ($R^2=0.9808$)