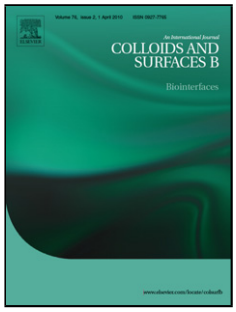


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# A thermosensitive drug delivery system prepared by blend electrospinning

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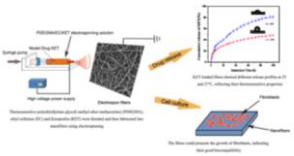
Graphical abstract



## Highlights

- 1. Thermosensitive PDEGMA was synthesized by free-radical polymerization.
- 2. PDEGMA and EC were blended and electrospun into nanofibers successfully.
- 3. The wettability of the EC/PDEGMA fibers changed as the temperature increased.
- 4. KET loaded-fibers showed different release behaviors at 25 and 37 ºC.
- 5. PDEGMA/EC fibers were found to have good biocompatibility towards fibroblasts.

**Abstract:**

In this study, the thermosensitive polymer poly(di(ethylene glycol) methyl ether methacrylate) (PDEGMA) was synthesized and electrospun into fibers by blending with ethyl cellulose (EC). Fibers were additionally prepared loaded with ketoprofen (KET) as a model drug. Smooth cylindrical fibers could generally be observed by electron microscopy, although there were some beads and fused fibers visible in the KET-loaded materials. KET was found to be amorphously distributed in the fibers on the basis of X-ray diffraction data. From water contact angle measurements, it was clear that the wettability of the EC/PDEGMA fibers changed as the temperature increased, with the fibers becoming markedly more hydrophobic. *In vitro* drug release studies showed that KET was released over a prolonged period of time with the fibers having different release profiles at 25 and 37 ºC, reflecting their thermosensitive properties. Furthermore, the materials were found to have good biocompatibility towards L929 fibroblasts. Thus, the fibers prepared in this work have potential as smart stimuli-responsive drug delivery systems.

Keywords: Drug delivery system; electrospinning; thermosensitive; poly(di(ethylene glycol) methyl ether methacrylate); ethyl cellulose.

## 1. Introduction:

New drug delivery systems (DDSs) have been studied intensively over many years [1]. An ideal formulation should be biocompatible, cost effective, and be able to deliver drug(s) at predetermined rates to achieve optimal therapeutic outcomes [2]. Stimuli-responsive materials have been extensively explored to this end. These permit a drug to be delivered in response to fluctuations in physiological parameters (e.g. temperature, pH) or the presence of certain biomolecules [3]. Many thermosensitive DDSs have been reported, with most based on temperature-responsive polymers. Poly(N-isopropylacrylamide) (PNIPAM), which has a lower critical solution temperature (LCST) of 32 °C, is at present the most studied thermoresponsive polymer [4,5], largely because it undergoes a hydrophilic/hydrophobic phase transition at a

temperature close to the physiological range. Various thermosensitive DDSs prepared from PNIPAM have been reported, including self-assembled micelles, hydrogels, nanoparticles, and nanofibers, among others [6-10].

Electrospun nanofibers have attracted much attention in the field of drug delivery, with researchers enticed by the technique's ability to rapidly produce amorphous solid dispersions with a wide range of polymers and active ingredients. Electrospinning is a relatively simple method to implement, and results in non-woven fiber mats with individual fibers having diameters typically on the nanoscale. With the recent development of high-throughput electrospinning techniques, it is possible to produce quantities of materials which can fulfil both the technological and capacity requirements of the pharmaceutical industry [11-13].

Electrospun fiber mats have high porosity, and their properties can be tuned through variation of the processing parameters. The fibers have high surface area-to-volume ratios, offering large areas for cell attachment. The mats also resemble the morphological structure of the extra-cellular matrix (ECM), and with judicious polymer choice exhibit good biocompatibility [14-16]. The selection of polymer carrier and the exact electrospinning technique employed can be used to control drug release [17].

A number of researchers have fabricated nanofibers based on PNIPAM *via* electrospinning, and the resultant materials show promising thermosensitivity and drug release behaviors. For example, Azarbayjani et al. prepared fibers from a blend of poly(vinyl alcohol) (PVA) and PNIPAM, using levothyroxine as a model drug. It was observed that the fibers were able to sustain the penetration of levothyroxine into the skin and help maintain an effective drug concentration over a prolonged period [18]. In other work, Lin and co-workers blended PNIPAM with poly(2-acrylamido-2-methylpropanesulfonic acid) to obtain composite fibers loaded with nifedipine. Drug release could effectively be controlled through adjustment of the temperature [19].

Beyond PNIPAM, in recent years another series of thermoresponsive polymers based on oligo(ethylene glycol) methyl ether methacrylate (OEGMA) has emerged. These polymers have graft structures composed of a carbon–carbon backbone and multiple oligo(ethylene glycol) side-chains [20]. They have been found to have a number of advantages over PNIPAM, including better bio-repellency below the LCST, reversible phase transitions without marked hysteresis, and bio-inert properties [4]. As a result, they are generally considered superior to PNIPAM-based formulations. However, few researchers have generated OEGMA-based fibers by electrospinning, largely owing to these polymers having poor electrospinnability and thus being very difficult to process alone.

In this work, we first synthesized an OEGMA-based polymer, poly(di(ethylene glycol) methyl ether methacrylate) (PDEGMA). This material has an LCST just below 30 °C; below this temperature, water interactions with hydrophilic segments of the polymer predominate, resulting in the polymer being linear and soluble. Above the LCST, polymer–polymer interactions are thermodynamically favored over polymer-water interactions, resulting in the polymer forming globules [21-23]. The change in interactions occurring at the LCST affects the hydrophilicity and wettability of the polymer, and thus if such a thermoresponsive polymer is used to form a scaffold for drug delivery very different release properties are to be expected: it is well known that hydrophobic systems tend to release their drug cargo more slowly than hydrophilic analogues.

Because PDEGMA alone is not amenable to electrospinning, it is necessary to blend it with a carrier polymer. For this we chose ethyl cellulose (EC), an inert, water-insoluble and non-toxic polymer that has been widely used to prepare sustained-release formulations, and has the benefit of very good electrospinnability [24, 25]. EC was selected based on previous work showing it to have promising biocompatibility and drug delivery behavior [26, 27]. Ketoprofen (KET), a non-steroidal anti-inflammatory drug, was selected as a model drug and mixed with a PDEGMA/EC solution to allow

the formation of composite fibers able to act as thermosensitive drug delivery systems. Morphological observations, physical form characterizations and *in vitro* drug release and biocompatibility assays were undertaken.

## 2. Experimental:

### 2.1 Materials

Di(ethylene glycol) methyl ether methacrylate (DEGMA, 95%), 1,1′-azobis(cyclohexanecarbonitrile) (VAZO-88, 98%), phosphate-buffered saline (PBS), penicillin, trypsin, and thiazolyl blue (MTT) were purchased from Sigma-Aldrich Ltd. (USA). Azobisisobutyronitrile (AIBN), anhydrous ethanol, acetone and n-hexane 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). L929 cells were provided by the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, China). Dimethyl sulfoxide (DMSO) and Dulbecco's Modified Eagle Medium (DMEM) 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 PDEGMA synthesis

PDEGMA was synthesized by free-radical polymerization, as detailed in our previous work [21]. Briefly, 1.88 g of DEGMA and 0.0025 g of the initiator VAZO-88 were dissolved in 2 mL of DMF, to give a molar ratio of DEGMA : VAZO-88 of 2000:1. Polymerization was undertaken at 90°C under a positive pressure of $N_2$. After 90 minutes, the resultant solid was dissolved in dichloromethane and PDEGMA precipitated through the addition of n-hexane. This dissolution/precipitation process was repeated five times. Finally the product was dried for 3 days in a vacuum oven (DZF-6050, Shanghai Laboratory Instrument Work Co. Ltd., China) prior to characterization and onward processing. Successful polymerization was evidenced by

$^{1}$H nuclear magnetic resonance (AV-400 instrument, Bruker, Germany) and Fourier transform infrared spectroscopy (FTIR; Nicolet-Nexus 670, Nicolet Instrument Corporation, USA). Gel permeation chromatography (GPC) measurements, performed on an LS measurement system (Waters, USA) with tetrahydrofuran as the solvent, were used to determine Mw, Mn and the molecular weight distributions. The flow rate and column temperature were 1.0 mL/min and 35 °C, respectively. Calibration was undertaken with polystyrene standards.

2.3 Preparation of electrospinning solutions

EC and PDEGMA were firstly dissolved in HFIP at room temperature, with magnetic stirring performed overnight to ensure complete dissolution. The component ratios of EC to PDEGMA were 1:1, 1:2 or 1:3 (w/w), and following a series of optimization experiments the total concentration of polymer was set at 15 % (w/v). Solutions of EC alone were also prepared as controls. KET was added into certain solutions at a drug to polymer ratio of 1:5 (w/w). Full details of all solutions prepared are listed in **Table 1**.

2.4 Electrospinning

The electrospinning solutions were loaded into 5.0 mL plastic syringes, which were fitted with a stainless steel needle (internal diameter 0.5 mm) and mounted on a syringe pump (KDS100, Cole-Parmer, USA). A flow rate of 0.8 mL/h was used during electrospinning, and fibers fabricated under a voltage of 14 kV (ZGF-2000 power supply, Shanghai Sute Electrical Co. Ltd., China). The grounded collector (a flat piece of aluminum foil of 10 × 10 cm in size) was placed 15 cm from the needle tip. Spinning was performed at ca. 40 % relative humidity, and at a temperature of approximately 25 $^{o}$C. After electrospinning for 8 hours, the products were stored in a vacuum oven at room temperature for 24 h, to remove any residual solvent.

2.5 Fiber characterization

Fiber morphology was studied using a scanning electron microscope (SEM; JSM-5600 LV microscope, JEOL, Japan) at a voltage of 10 kV. Samples were first 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 via the analysis of approximately 100 fibers in the SEM images.

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

The water contact angle (CA) of each fiber mat was determined on a contact angle analyzer (DSA 30, Krüss GmbH, Germany) in air. A water droplet (ca. 5 µL) was placed onto the surface of the fibers and the CA recorded. The measurement temperature was varied from 20 to 45 ºC using a heating platform (XMTD-204, JTHF Company, China). Five measurements were recorded for each sample, and the results are reported as mean ± S.D.

2.6 *In vitro* drug release

Drug release experiments were conducted at two different temperatures (25 or 37 ºC) 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 (pH 7.4 PBS). At predetermined time points, 1 mL of the test medium was withdrawn and an equal amount of fresh preheated PBS (at either 25 or 37 ºC) was 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 Cell growth

To prepare samples for *in vitro* cellular toxicity tests, fibers were electrospun directly onto cover slips. 20 cover slips were placed onto the collector plate and 5 mL of spinning solution dispensed onto them. The fiber-covered slips were subsequently placed in the wells of 24-well plates, with untreated cover slips used as a negative control. The plates were sterilized with alcohol steam for 24 h. For cell culture, 400 μL of dissociated L929 fibroblasts ($1.0 \times 10^4$ cells/mL, in DMEM supplemented with 10 % v/v FBS and 1 % v/v penicillin–streptomycin) was added into each well, and the plates cultured in an incubator (37 ºC, 5 % $CO_2$). After 1, 3, or 5 days, the culture medium in each well was replaced by 360 μL of fresh DMEM and 40 μL of an MTT solution (5 mg/mL thiazolyl blue in PBS). The plates were shaken for 30 min at room temperature, before 400 μL DMSO was added to each well and the plates incubated for 6 h. The resultant purple solution in each well was transferred to a 96-well plate and the number of cells assessed via the optical density (OD) values at 570 nm, which were quantified on a microplate reader (Multiskan, ThermoFisher, USA).

**3. Results and discussion:**

3.1 Synthesis of PDEGMA

Both the FTIR and $^1$H NMR spectra verify the successful polymerization of DEGMA. In the FTIR spectra (Supplementary Materials, **Figure S1**), it can be seen that the DEGMA monomer shows a C=C stretching vibration at 1638 $cm^{-1}$, which disappears in the spectrum of the polymer. The $^1$H NMR spectrum of DEGMA (**Figure S2**(a); $D_2O$, 400 MHz) shows resonances as follows: δ (ppm): 5.99 (1H, C**H**$_2$=C), 5.55 (1H, C**H**$_2$=C), 4.16 (2H, -C**H**$_2$-), 3.68 (2H, -C**H**$_2$-), 3.58 (2H, -C**H**$_2$-), 3.48 (2H, -C**H**$_2$-), 3.21 (3H, -OC**H**$_3$), 1.80 (3H, $CH_2$=C-C**H**$_3$). The spectrum of PDEGMA, shown in **Figure S2**(b), reveals the absence of vinyl groups. The signals at δ of 5.99 (1H, C**H**$_2$=C), 5.55 (1H, C**H**$_2$=C) of DEGMA have disappeared, confirming that PDEGMA was

successfully obtained. The molecular weights (Mw and Mn) and molecular weight distribution of PEDGMA were determined by GPC to be 35,573, 29,734, and 1.19, respectively.

3.2 Fiber production

Prior to blending PDEGMA with EC, attempts were made to prepare fibers of PDEGMA alone. PDEGMA could not be dissolved in most of the common solvents used for electrospinning (see Supplementary Materials, Table S1), and only HFIP proved able to dissolve PDEGMA to yield a solution of appropriate viscosity for spinning. However, when this solution was electrospun, only droplets and micron-sized particles were obtained (see Figure S3 and S4). EC was therefore added to increase the spinnability of the solution.

SEM images of all the fibers prepared, together with their diameter distributions, are depicted in **Figure 1**. In all cases, with the inclusion of EC fibers have been successfully fabricated. The average diameter of pure EC fibers (S1) is 960 ± 215 nm. In comparison, the EC fibers loaded with KET (S5) have a narrower average diameter (556 ± 145 nm). A similar phenomenon can be seen with the other materials; the KET-containing fibers always have smaller diameters than their drug-free analogues. The addition of KET thus decreases the fiber diameters, as a result of the solutions having increased conductivity when the drug is added [28]. This can extend the elongation of the polymer jet and thereby generate narrower fibers. The EC/KET S5 fibers also show some beading.

Considering the blend fibers, S2 and S6, which have the greatest content of PDEGMA (1:1 w/w PDEGMA: EC ratio), show non-uniform and fused fibers, which might be explained by the poor spinnability of PDEGMA. With an increase in the EC concentration (to a 1:2 PDEGMA: EC w/w ratio), cylindrical and liner fibers can be observed in the cases of S3 (461 ± 196 nm) and S7 (413 ± 150 nm), although two distinct fiber populations can be seen. A number of very narrow fibers are mixed with

fibers having much larger diameters. Some bead-on-string morphology can also be seen for S7. In comparison, S4 and S8 (1:3 PDEGMA: EC) have more consistent morphologies. Fewer fibers with very small diameters can be seen, and the linear fibers with sizes around 1000 nm are obtained for these formulations.

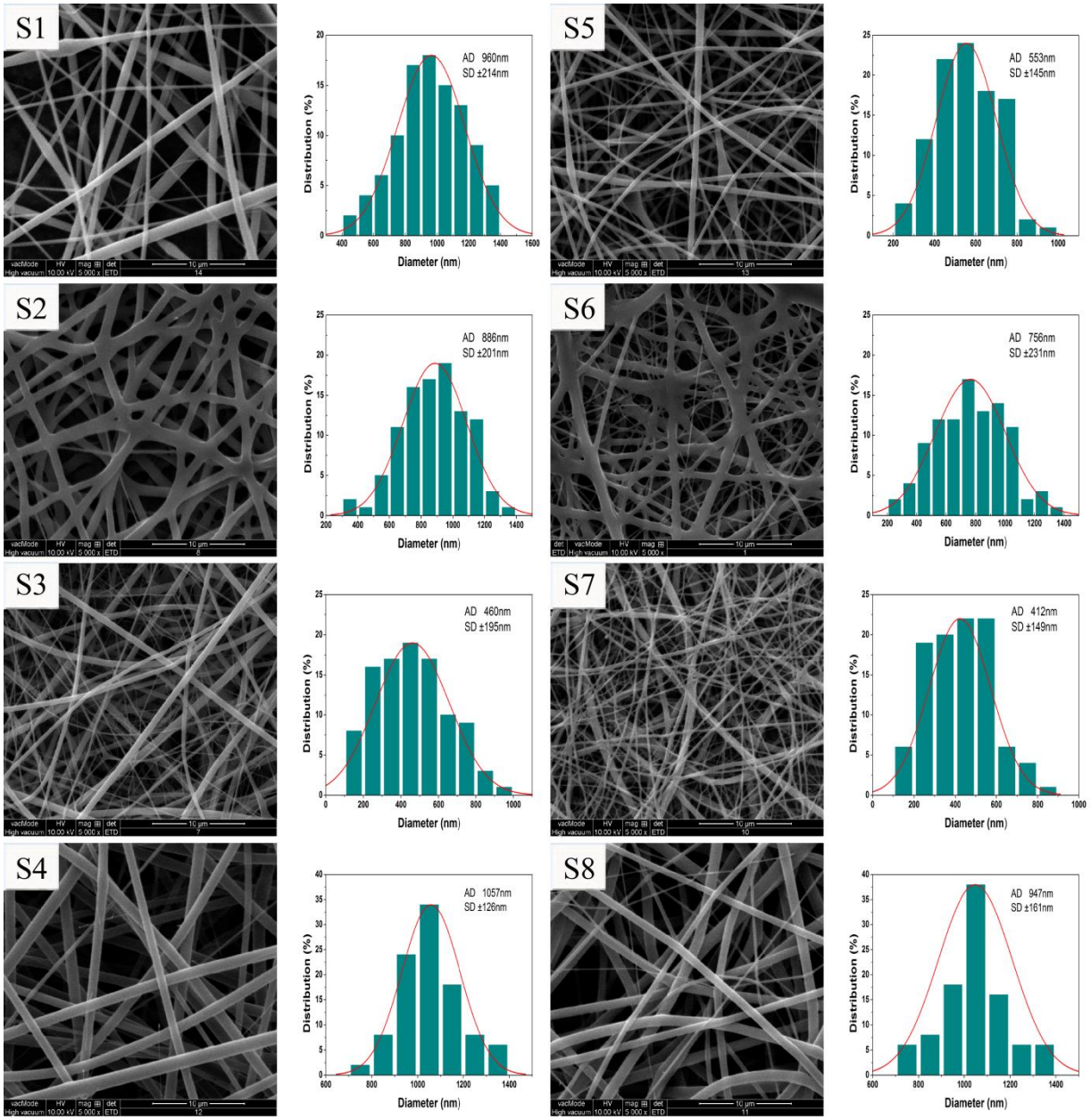



**Figure 1.** SEM images and diameter distributions of the fibers. The scale bar in the SEM images represents 10 µm.

3.3 X-ray diffraction

XRD patterns of all the fibers (S1 - S8) and pure KET are shown in **Figure 2(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 it to be a crystalline material, as has been reported previously in the literature [29]. In contrast, for all the fibers, only two weak and broad diffuse peaks at around 7º and 22º 2θ can be seen in the diffraction patterns. The fibers are hence all amorphous. The reflections of KET have disappeared in the KET-loaded fibers, indicating they comprise amorphous solid dispersions. Similar results have been reported in many previous studies in the literature [30-32]. Solvent evaporation during the electrospinning process is very rapid, which means that during drying there is insufficient time for the molecular organization required to form a crystal lattice [33, 34]. The random arrangement of molecules in the solution phase is thus propagated into the solid state, leading to the KET molecules being molecularly dispersed in the fibers.

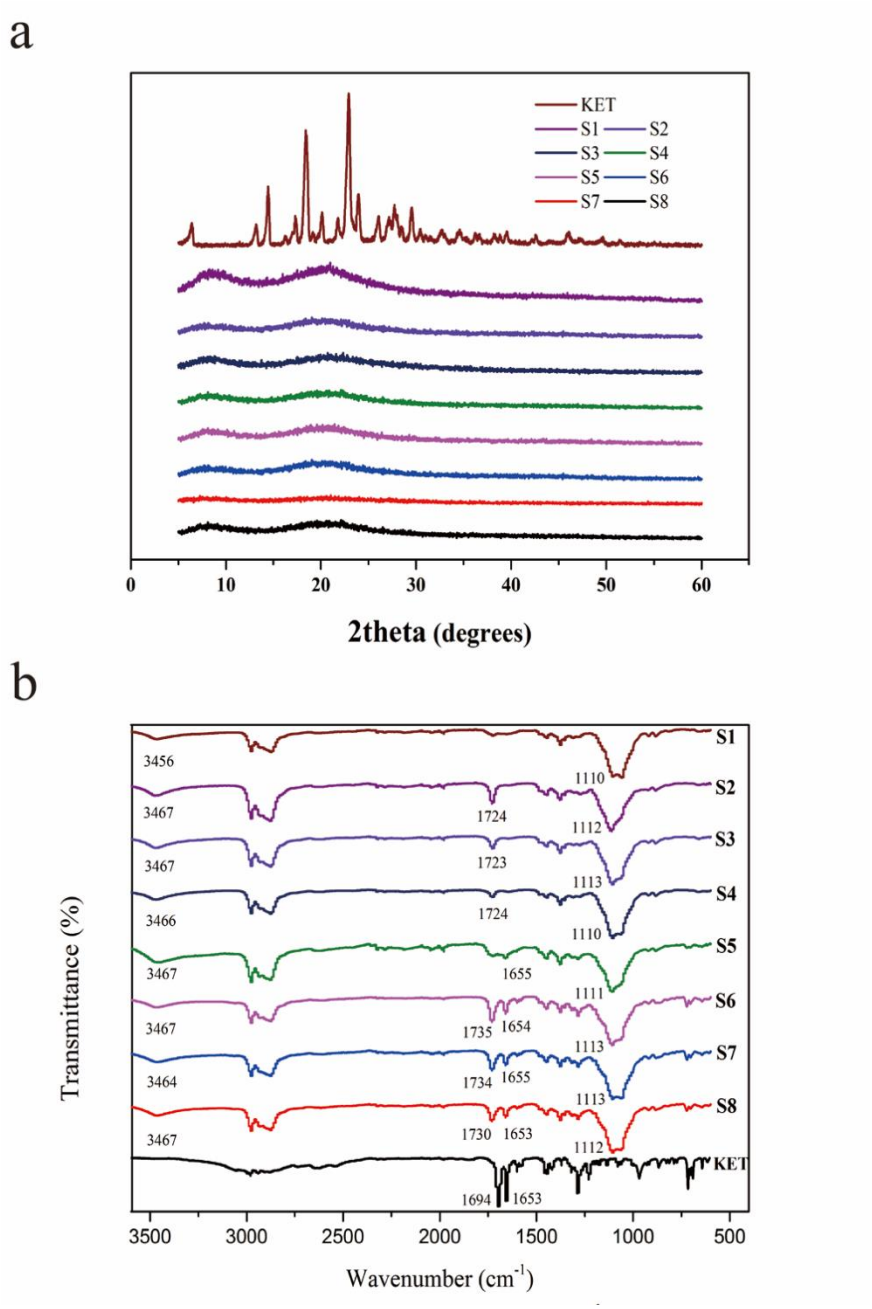



**Figure 2.** a) XRD patterns and b) FTIR spectra of the fibers and pure KET.

3.4 FTIR spectroscopy

FITR spectra of all the fiber samples and KET are given in **Figure 2(b)**. The neat EC fibers (S1) exhibit characteristic peaks arising from OH group vibrations (3456 $cm^{-1}$), C-H stretches (2750-3000 $cm^{-1}$) and C-O-C stretches (1110 $cm^{-1}$) [26]. For the drug-free composite fibers (S2-S4), peaks at 1723 or 1724 $cm^{-1}$ corresponding to the C=O

stretching of PDEGMA are also visible, confirming the presence of both polymers. In addition, some small shifts in the peak positions of the OH absorbances can be seen. This peak arises at 3456 $cm^{-1}$ for S1, but is shifted to 3466 $cm^{-1}$ (S4) or 3467 $cm^{-1}$ (S2 and S3), indicating the presence of intermolecular interactions between EC and PDEGMA.

Considering the pure KET spectrum, two distinct peaks at 1694 and 1653 $cm^{-1}$ represent the stretching vibration of the carbonyl group and stretching of the ketone group, respectively. The peak at 1653 $cm^{-1}$ can be observed in the drug-loaded samples (S5-S8), confirming the incorporation of KET into the fibers. However, the peak at 1694 $cm^{-1}$ cannot be seen in the spectra of fibers S5 – S8. 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 1694 $cm^{-1}$ [35, 36]. Therefore, 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.

3.5 Thermosensitive behavior

The thermosensitive behavior of the composite fibers was explored by measuring water contact angles as a function of temperature. As shown in **Figure 3**, the CAs of S5 (EC/KET fibers) are largely constant at around 136º, indicating that these fibers are hydrophobic at all temperatures studied. In contrast, the PDEGMA/EC fibers have wettability which changes with temperature. The CA of S8 increases from 67.5 ± 4.5º at 20 ºC to 128.8 ± 5.8º at 45 ºC. In comparison, the CAs of S6 at 20 ºC and 45 ºC were around 0 º and 109.7 ± 4.5 º, respectively. It is clear that fibers with a greater proportion of PDEGMA have more profound changes of CA with temperature.

The CAs of the composite fibers change most rapidly at around 30 ºC, which is close to the LCST of pure PDEGMA. It is expected that the PDEGMA and PDEGMA/EC fibers will have similar temperature-dependent interactions with water. The PDEGMA

molecules in the fibers will have favorable interactions with water when the temperature is below 30 ºC, because interactions between water molecules and hydrophilic components of the polymer outweigh hydrophobic considerations. Thus, a water droplet placed on the fiber surface will spread across it to maximize these favorable hydrophilic interactions. The balance will tip in the other direction when the temperature rises above the 30 ºC, and hydrophobic-hydrophobic interactions between different segments of the polymer will become more important. These will repel an incoming water droplet, causing an increase in the CAs.

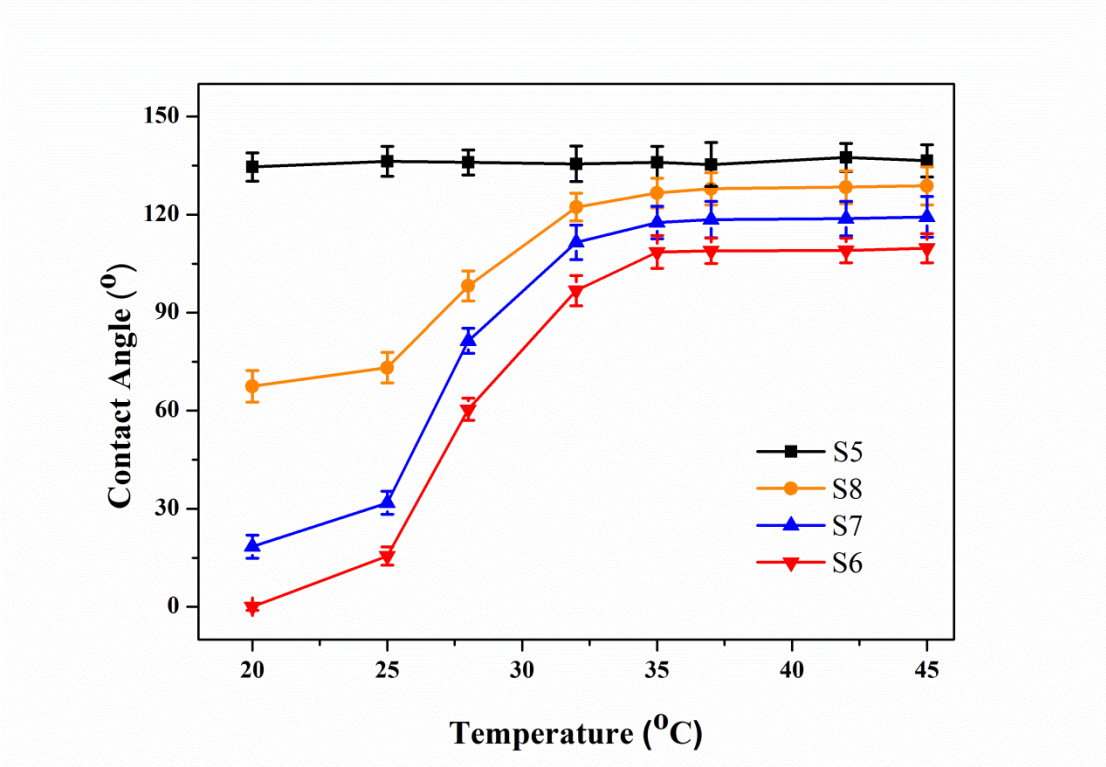



**Figure 3.** Water contact angles of S5, S6, S7 and S8 as a function of temperature. Data are reported as mean ± S.D. from three independent experiments.

3.6 Drug release

The results of *in vitro* release studies are given in **Figure 4**. Experiments were performed at two different temperatures, 25 and 37 ºC. For the S5 fibers (EC/KET), the release behavior is very similar at both temperatures. After an initial burst release of 15 % release in the first 10 hours, the fibers showed gradual release over ca. 90 hours. By

the end of the experiment (100 hours), the cumulative release of KET at 25 and 37 ºC reached around 42 %. These slow release behaviors can be explained by the hydrophobic and insoluble nature of EC. Even after immersion for 100 hours, some KET was still encapsulated deep inside the EC fibers and could not diffuse to the release medium during the experiment.

The composite PDEGMA/EC fibers S6, S7 and S8 show thermosensitive KET release. At 37 ºC they have similar behavior to S5. After 100 hours, the cumulative release for S6, S7 and S8 reached 48.0, 45.8 and 42.3 %, respectively. This is because all the fibers, with or without PDEGMA, have similar hydrophobic properties at this temperature.

Considering the behavior at 25 ºC, faster release rates are observed and higher cumulative release percentages reached at the end of the experiment. S6 gave the greatest amount of release of KET, followed by S7 and S8. These different release behaviors are a result of the fibers' different surface wettability: the release rate from a hydrophilic carrier tends to be faster than that from a hydrophobic material [19]. S6 has the greatest proportion of PDEGMA and also displayed the highest wettability of the three samples at 25 $^{O}$C, followed by S7, and S8. These trend mirrors the order of release rates (S6 > S7 > S8). The cumulative release of KET from S6, S7 and S8 at 25 ºC reaches 80.8 %, 75.5 % and 71.0 %, respectively; all of these are much higher than the values observed at 37 $^{O}$C.

A thermosensitive drug delivery system where release slows upon a rise in temperature could be useful for conditions causing a rise in body temperature, although the polymer LCST would need to be closer to (slightly above) the normal physiological temperature than is the case here. For example, diabetic patients could be treated by a system which releases insulin at the normal body temperature (37 ºC). The rise in insulin concentration in the blood tends to cause an increase in body temperature. If the body temperature rises beyond a certain limit, the system would pass through its LCST and cease to release drug. This could be very beneficial, because the insulin-driven temperature rise triggers detrimental burning of "brown fat" cells in the body and a loss

of appetite, which can in turn lead to a risk of hypoglycemia. By using a thermosensitive drug release system similar to those produced in this work, patients could be supplied with a self-regulating implant. Furthermore, LCST systems could also be used in other conditions where patients suffer from high body temperatures, such as inflammation and fever. With higher body temperatures, the metabolic rates of patients will be slower, and reduced rates of drug release will be needed.

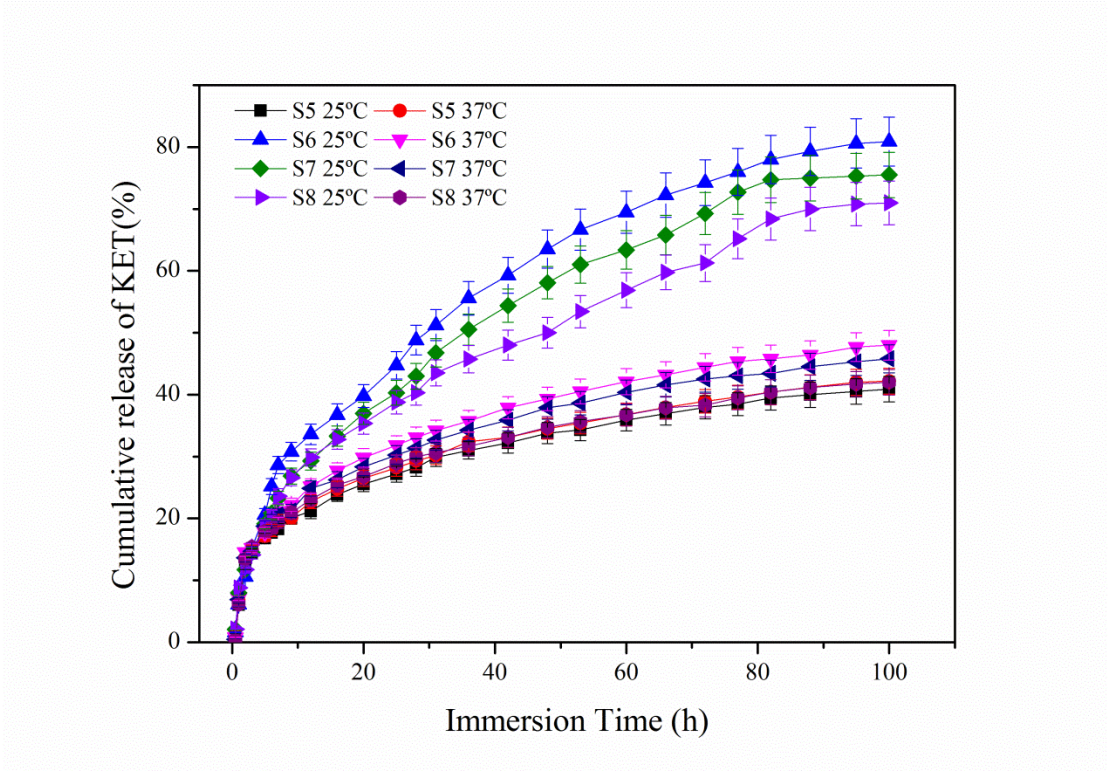



**Figure 4.** The *in vitro* release profile of KET from S5, S6, S7 and S8. Data are reported as mean ± S.D. from three independent experiments.

The mechanism of drug release was explored using the Peppas equation [37]:

$Q = kt^n$

where Q is the percentage of drug released at a given time, t is the release time, k is a rate constant, and n is an exponent that indicates the drug release mechanism. The outcomes of this analysis are summarised in **Table 2**, with plots included in the

Supplementary Materials, Figures S5-S12. The release exponents for S6, S7 and S8 at 25 ºC are 0.499, 0.502 and 0.452, respectively. These values are larger than 0.45 but smaller than 1.0, indicating that KET was released through a combined mechanism of polymer erosion and drug diffusion [38]. When the temperature is 37 ºC the exponents decrease to 0.338, 0.333 and 0.317. These exponents are smaller than 0.45, suggesting that a typical Fickian diffusion mechanism is dominant at the higher temperature. Therefore, the drug release mechanisms of the PDEGMA/EC fibers are different at the two temperatures explored, as a result of the thermoresponsive behavior of PDEGMA. At 25 ºC, PDEGMA is hydrophilic and thus can dissolve out of the fibers, leading to erosion. The latter will create pores in the fibers and aid drug release. However, at 37 ºC the PDEGMA is hydrophobic and insoluble, and the KET must diffuse through the polymer matrix to reach the release medium.

3.7 Biocompatibility

The results of MTT assays are depicted in **Figure 5**. It can be seen that L929 cells could grow and proliferate on coverslips coated with all the different fibers over 5 days. There are no clear differences in cell viability, but there appear to be a slightly greater number of cells on the blank coverslips than with the fiber groups (S5, S6, S7 and S8) on the 3$^{rd}$ day of culture. After culture for 5 days, S5 (neat EC fibers load with KET) showed the greatest cell viability. The excellent biocompatibility of EC/KET fibers has also been observed in our previous work [26], where we found that EC-based fibers are more suitable for cell growth than similar EC/PNIPAM materials. Here, cell viability did not decrease significantly when the cells were cultured on the composite PDEGMA/EC fibers, indicating that PDEGMA has similar biocompatibility to EC and may have greater potential than PNIPAM in the production of thermoresponsive drug delivery systems.

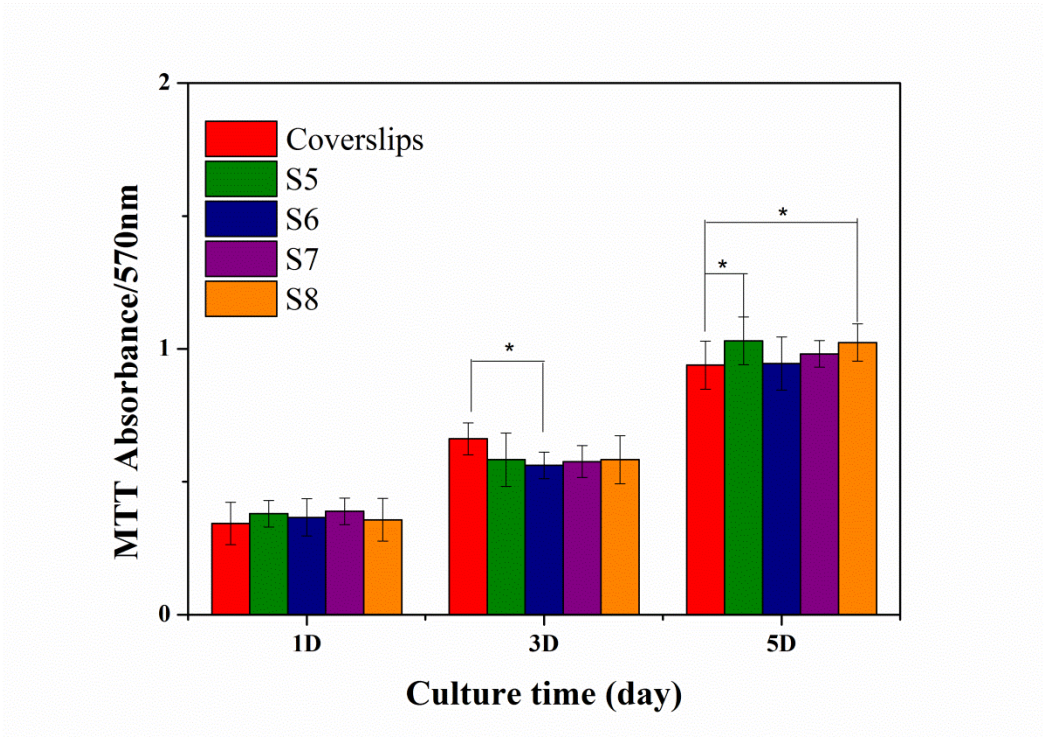



**Figure 5.** Cell viability after exposure to S5, S6, S7 and S8 fibers for 1, 3, or 5 days. Blank coverslips were used as a negative control. Data are shown as mean ± S.D. from 3 independent experiments. * indicates a significant difference between two groups ($p < 0.05$).

**4. Conclusions:**

In this work, the thermoresponsive polymer poly(di(ethylene glycol) methyl ether methacrylate) (PDEGMA) was synthesized by free-radical polymerization. Thermosensitive fibers based on blends of PDEGMA and ethyl cellulose (EC) were then prepared by electrospinning, with both blank polymer fibers and those loaded with the model drug ketoprofen (KET) prepared. Electron microscopy images showed the fibers to have regular morphology and diameters between 400 and 1050 nm. Some beads or fused fibers were visible with the KET-loaded fibers. X-ray diffraction and infrared spectroscopy revealed that KET was present in the amorphous state in the fibers. The water contact angles of the PDEGMA/EC fibers increased dramatically as the temperature was raised through the lower critical solution temperature of PDEGMA (30 ºC). *In vitro* drug release studies showed that KET could be released over around

100 h, with different release behaviors and mechanisms observed at 25 ºC and 37 ºC for the PDEGMA/EC fibers. MTT assays using the L929 cell line revealed the fibers to have good biocompatibility. PDEGMA/EC fibers thus have potential as effective and biocompatible thermoresponsive materials for use in drug delivery systems and tissue engineering.

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