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Yao Lv[1], Qixia Pan[1], S. W. Annie Bligh[2*], Heyu Li[1], Huanling Wu[1], Qingqing Sang[1], Li-Min Zhu[1*]

1. College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai, 201620, China
2. Department of Life Sciences, Faculty of Science and Technology, University of Westminster, 115 New Cavendish Street, London W1W 6UW, UK

* Authors for correspondence. Email: lzhu@dhu.edu.cn (LMZ); a.bligh@westminster.ac.uk (SWAB).

Abstract

Thermoresponsive, polymer-based core-sheath nanofibres are of great interest as advanced materials because they are capable of responding to external stimuli and delivering drugs as part of release strategy. Core-sheath nanofibers were constructed by using thermoresponsive poly-(N-isopropylacrylamide) (PNIPAAm) (as core) and hydrophobic ethylcellulose (EC) (as sheath) by coaxial electrospinning. Analogous medicated nanofibers were prepared by loading with a model drug ketoprofen (KET). The fibers were cylindrical without phase separation and have visible core-sheath structure as shown by scanning and transmission electron microscopy. X-ray diffraction patterns demonstrated the drug with the amorphous physical form was present in the fiber matrix. Fourier transform infrared spectroscopy analysis was conducted, finding that there were significant intermolecular interactions between KET and the polymers. Water contact angle measurements proved that the hydrophilic hydrophobic transformation of core-sheath fibers had taken place when the temperature reached the lower critical solution temperature. *In vitro* drug-release study of nanofibers with KET displayed that the coaxial nanofibers were able to synergistically combine the characteristics of the two polymers producing a temperature-sensitive drug delivery system with sustained release properties. In addition, they were established to be non-toxic and suitable for cell growth. These findings show that the core–sheath nanofiber is a potential candidate for controlled drug delivery system.

Keywords: poly(N-isopropylacrylamide), ethylcellulose, ketoprofen, coaxial electrospinning, controlled drug release

1. Introduction

Electrospinning has attracted much attention in fabricating nanofibers in nanotechnology. The characteristics of electrospun nanofibers, including high surface area to volume ratio, small diameters, and high porosity, have made the nanofibers useful in biomedical applications, such as wound healing, drug delivery, and scaffolding for tissue engineering.[1-3] Coaxial electrospinning, comparing to simple single and blended spinning, is used to prepare core-sheath structured nanofibers with outstanding properties and multifunctionality for additional functional applications,[4-5] such as biotechnology, textiles, membranes, composites.[6-9]

Stimuli-responsive polymers have been widely used in intelligent or smart materials with a great promise in drug delivery systems, sensors, and biomaterials,[10-15] due to these polymers can respond to changes in temperature, pH, light, or ionic strength. For example, poly(N-isopropylacrylamide) (PNIPAAm) is a typical temperature-sensitive polymer. It undergoes a sharp phase transition between hydrophilicity and hydrophobicity at a lower critical solution temperature (LCST) of 32ºC.[16-17] When the temperature is raised from below to above the LCST, PNIPAAm rapidly changes from being hydrophilic to hydrophobic.[18-19] Thus, due to its thermoresponsive property, the PNIPAAm polymer has been studied intensively for its potential applications in biomedicine.[20-25]

PNIPAAm nanofibers cannot be electrospun easily because of it being thermo-sensitive. Thus, for aiding the spinning process and enhancing the quality of the fibers, researchers have attempted to produce blends of PNIPAAm with other polymers, or coaxial nanofibers with PNIPAAm as core and other polymers as sheath.[26-27] Previous reports showed that PNIPAAm and poly(L-lactide) (PLLA) mixture with tunable surface morphologies can be increased by spinning from N-N-dimethylformamide (DMF).[28]

Ethyl cellulose (EC) is a kind of polysaccharides with a good film-forming ability, hydrophobicity and biocompatibility. Therefore, it is widely used in nanofibers

preparation, either blended or coaxial electrospun, for application in drug- release fields.[29] Hu et al. have employed the blended electrospun of PNIPAAm and EC to prepare nanofibers for delivering drug. This delivery system has advantages in drug sustained release and biocompatibility. It gives a burst release when the temperature is under the LCST and changes to slow release when the temperature is above the LCST. To increase drug-sustained delivery and improve the biocompatibility, the method of preparation was then optimized by coaxial electrospinning. [30]

Ketoprofen (KET), a non-steroidal anti-inflammatory drug, has been widely used for the treatment of inflammation, pain and rheumatism but has a short biological half-life (1.5 to 2 hours).[31] It also has a poor solubility in water (0.5 μg/mL).[32] Thus, it is necessary to develop a controlled release delivery of KET to increase its efficiency and reduce its side effects.

In this study, a controlled released system based on PNIPAAm and loaded with KET was developed by coaxial electrospining. Here, we aim to prepare thermoresponsive controlled drug release coaxial nanofibers using PNIPAAm and EC, and to characterize these fibers by scanning electron microscopy (SEM), transmission electron microscope (TEM), Fourier transform infrared (FTIR), X-ray diffraction (XRD). The coaxial nanofibers were tested for its a thermoresponsive phase change using water contact angle (CA) measurements. Medicated coaxial and blended nanofibers with KET were prepared and tested for their drug-controlled release. They were assayed for their biocompatibility using MTT tests.

2. Experimental Part

2.1 Materials and methods

N-isopropylacrylamide (NIPAAm, Tokyo Chemical Industry Co. Ltd., Tokyo, Japan, 98%) was recrystallized from n-hexane. Ethy cellulose (EC) was purchased from the Aladdin Chemistry Co. Ltd. (Shanghai, China). Ketoprofen (KET) was obtained from Beijing JK Scientific Co., Ltd. (Beijing, China). L929 cells (a mouse fibroblast cell line) were purchased from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, Shanghai China). DMEM medium, thiazolyl

blue (MTT reagent), fetal bovine serum (FBS), phosphate-buffered saline (PBS), penicillin and streptomycin were supplied by the Nanjing keyGEN Biotechnology Co., Ltd. (Nanjing, China). Dimethyl sulfoxide (DMSO) was purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Acetone was purchased from the Yonghua Chemical Technology Co., Ltd. (Suzhou, China). Azobisisobutyronitrile (AIBN) was obtained from Shisi Hewei Chemical (Shanghai, China). Anhydrous ether and anhydrous ethanol were obtained from Changshu Hongsheng Fine Chemical (Suzhou, China). All chemicals were of analytical grade and used directly without further purification. Water was deionized prior to use.

2.2 Synthesis and purification of PNIPAAm

PNIPAAm was synthesized according to the simple atactic copolymerization as described in the literature.[30] NIPAAm (5.0 g) and AIBN (25.0 mg) were dissolved in anhydrous ethanol (10 mL) and heated to 70ºC under a position pressure of N_2. The resultant polymer was precipitated in anhydrous ether after 7 h. Finally, pure product was obtained through precipitating from acetone into anhydrous ether three times.

2.3 Preparation of coaxial nanofibers

Two different kinds of electrospinning solutions were prepared to fabricate the coaxial nanofibers. The shell solution was composed of EC and anhydrous ethanol under magnetic stirring at room temperature for 24 h. Meanwhile，the core solution was prepared by dissolving PNIPAAm in anhydrous ethanol at room temperature for 24 h. The ratio of PNIPAAm and EC were set as 25% (w/v) in the total polymer concentration. In addition, the PNIPAAm/EC blend nanofibers were also prepared in this work. The component ratio of PNIPAAm to EC was 1:2 (w/w), and the total concentration of polymer was 25 % (w/v). Solutions containing 25 % (w/v) of PNIPAAm alone was also prepared as controls. KET was added into core solution at a drug to polymer ratio of 1:4 (w/w).

The electrospinning solution were placed into a 5 mL plastic syringe fitted with a special metallic needle (Fig. 1a: inner diameter of inner tube 0.34 mm, inner diameter of outer tube 1.12 mm), and the syringe was fixed horizontally on a syringe pump (KDS100, KD Scientific), and an electrode of high voltage power supply (Spellman

High Voltage Electronics Corporation, MP Series) was clamped to the metal needle, as shown in Fig. 1b. During the spinning process, the flow rate of the shell solution was maintained at 0.4 mL/h, which was the same as the core and the blending solutions. The applied voltage was 9-11 kV and the tip-to-collector distance was set at 10 cm. The last nanofiber was collected by a piece of clean aluminum foil, and then dried at 25ºC in a vacuum oven for 24 h to remove any residual solvent. There six kinds fibers were prepared: pure PNIPAAm nanofibers(A1), blend nanofibers (A2), coaxial nanofibers (A3), and pure PNIPAAm nanofibers with KET(A4), blend nanofibers with KET (A5), coaxial nanofibers with KET (A6). Full details of all solutions are listed in Table 1.

（Figure 1）

(Table 1)

2.4 Nanofibers Characterization

After plating the coating with an ion sputter, the fibers morphological features were analyzed by scanning electron microscopy (SEM; JSM-5600 LV microscope, JEOL, Tokyo, Japan). The mean fiber diameter for each sample was calculated by measuring approximately 50 fibers in SEM images using the Image J software (National Institutes of Health, Bethesda, MD, USA). In addition, the coaxial fibers were also observed by a transmission electron microscope (TEM, H-800 instrument, Hitachi, Tokyo, Japan).

Fourier transform infrared spectroscopy (FT-IR) was conducted using a Nicolet-Nexus 670 spectrometer (Nicolet Instrument Corporation) over the range 4000–500 cm^{-1} and with a resolution of 2 cm^{-1}. X-ray diffraction (XRD) was undertaken on a D/max-BR diffractometer (Rigaku, Japan) with CuKα radiation (40 kV/20 mA) over the 2θ range 5º to 60º.

2.5 Thermoresponsive behavior

The water contact angle (CA) was determined by the sessile drop method with a pure water droplet (ca. 2 μL) using on a contact angle analyzer (DSA 30, Germany). A static image was taken by dropping deionized water onto the flat nanofibrous mats after 2 s. The temperature-controlled experiments were performed using a custom-made heating plate with a PID-controller. The mean value of five

measurements made at different surface locations on the same sample was adopted as the contact angle.

2.6 *In vitro* drug release

In order to study *in vitro* drug release, the drug-loaded nanofibers (100 mg) were immersed into a centrifuge tube filled with 20 mL phosphate buffer saline (PBS, pH 7.4) to monitor the drug-release behavior at 25 and also at 37ºC at a shaking rate of 90 rpm. For measuring the drug-release amount, 1 mL of KET released sample buffer was withdrawn and replaced with 1 mL of fresh buffer solution. The concentration of KET was analyzed by UV–Visible spectrophotometer (JingHua UV-1800) at a wavelength of 265 nm. The amount of KET released was determined using a standard calibration curve. All results were reported as mean values.

2.7 Cell viability assay

L929 fibroblasts cells were culture in DMEM medium supplemented with penicillin (100 units/mL), streptomycin (100 μg/mL), and 10%(v/v) heat-inactivated FBS. All the fibers need sterilize on the vapour of ethyl alcohol with 24 h. The pretreatment fibers were putted in the 24-well plates and then seed 400 μL of L929 cells into it. The number of cells were about 1×10^4 cells per well. After 1, 3 or 5 days incubation (37ºC， 5% CO_2), the culture medium in each well was removed and added with 360 μL of fresh DMEM and 40 μL of MTT solution. After incubation for 4 h, the medium solution was removed and replaced 400 μL DMSO. Each plate was shaken for 30 min at 37ºC. The resulting solution was transferred into a 96 plate, and then measured using a microplate reader (Thermo, Mnltiskan FC) at a wavelength of 570 nm.

3. Results and discussion

3.1 Preparation and characterization of PNIPAAm

PNIPAAm was characterized by NMR and IR and the data were compared with the literature data.[33] The FTIR spectrum of NIPAAm in Fig. 2a shows a sharp band at 1621 cm^{-1} (C=C stretching vibration), which had disappeared in the spectra of PNIPAAm. The chemical structure of the NIPAAm and PNIPAAm were confirmed by 1-H NMR as shown in Fig. 2b. The peaks at around 6.05 (qd, 2H, 2-H) and 5.56 (m, 1H, 4-H)

were attributed to C=C bond in NIPAAm while disappeared in PNIPAAm, which indicated the PNIPAAm has been synthesized successfully.

（Figure 2）

3.2 Nanofibres morphology

The morphology of all fibers was described by scanning electron microscopy (SEM) and the coaxial nanofiber was recorded by transmission electron microscopy (TEM), as presented in Fig. 3. Smooth and finer homogeneous nanofibers are shown in the A1, A2, A4, A5, which indicates that the blend and pure PNIPAAM fibers were prepared successfully. In addition, the diameters of coaxial nanofibers were calculated using Image J and the report data was mean values. Fig.3c and d show the coaxial electrospun fibers with the mean diameter of 380.5±110.81 and 474.93±127.09 nm, respectively. The diameter of the coaxial nanofibers increased after the loading of KET in the core fiber.

Further, transmission electron microscopy (TEM) was conducted to confirm the morphology of core-sheath nanofibers. In Fig. 4e, the sheath structure can be seen clearly by the different lightness of polymers caused by their different electron transmissivity. The dark place was PNIPAAm as core, while the bright place was EC as sheath. The diameters of fibers with loaded KET (Fig. 4f) were larger than fibers with drug-free, which is consistent with the diameters analysis of SEM. There are no black spots on the surface of the KET-loaded fibers as well as the KET-free fibers. TEM images indicated that drug was dispersed uniformly along the nanofibers and was not separated from the drug loaded fibers during solvent evaporation.

（Figure 3）

3.3 X-ray diffraction

The formations of the KET-loaded fibers were confirmed via X-ray diffraction. The patterns of pure KET demonstrate its crystalline nature, which show well-defined Bragg reflection at 6.3º, 13.1º, 14.4º, 18.4º, 19.2º, 20.1º, 21.7º, 22.9º, 23.9º, 26.0º and 29.5º. PNIPAAm nanofibers have two broad diffuse feature centered at around 8.7º and 22.0º, which conforms to amorphous physical form.[34] Comparing to the KET-free coaxial nanofibers, the KET-loaded coaxial materials additionally display

no Bragg reflections, which suggest that the drug has been changed to the amorphous through electrospinning.

（Figure 4）

3.4 IR spectroscopy

Fig. 5 shows the FTIR spectra of KET and all the nanofibers. The neat PNIPAAm nanofibers display a broad band between *ca.* 3100 and 3600 cm^{-1}, which corresponds to the superposition of H-bonded N-H stretches (3294 cm^{-1}). There also has a series of absorptions at 2750 – 3000 cm^{-1} arising from CH_3, CH_2 and CH stretches. Furthermore, characteristic peaks at 1650 cm^{-1} (C=O stretching vibration) and 1550 cm^{-1} (C-N stretching and N-H bending vibrations) were also observed. The peaks at lower wavenumbers are attributed to CH_3, CH_2 and CH bends. The characteristic peaks of PNIPAAm can also be seen in the blended (A2, 1110 cm^{-1}) and coaxial nanofibers (A3, 1108 cm^{-1}), which is caused by C-O-C stretching of EC.[35]

FTIR spectrum of KET displays characteristic peaks at 1695 cm^{-1} and 1655 cm^{-1} corresponding to C=O stretching vibration and C-O-C stretching, respectively. The peak at 1655 cm^{-1} is present in the spectra of A4, A5 and A6, which indicates the presence of KET in these nanofibers. It is however shifted somewhat: in A4, this peak is merged with the PNIPAAm C=O stretch into a single peak at 1645 cm^{-1}, and in A5 is moved to 1654 cm^{-1}. The peak at 1695 cm^{-1} cannot be seen in the spectra of A4, A5 and A6, indicating the absence of any KET dimers in the fibers. This is consistent with the result of X-ray diffraction analysis, which showed the absence of any crystalline KET.

(Figure 5)

3.5 Thermoresponsive behavior

The change of the responsive wettability of the nanofibers with temperature from 20ºC to 45ºC was studied in detail and is shown in Fig. 6. The water contact angle of pure PNIPAAm (A1) was approximately 0º when the temperature below 30ºC, while the water contact angle of blended nanofibers (A2) were about 20º and the coaxial nanofibers (A3) were about 60º, which was due to the hydrophobicity of the EC homopolymer. The blended nanofibers have a better hydrophilic property than coaxial

nanofibers because EC being the shell on the coaxial nanofibers surface makes it more hydrophobic. PNIPAAm is a stimuli-responsive polymer with the lower critical solution temperature (LCST) of about 32±2ºC.[36] The transformation of hydrophilic to hydrophobic of PNIPAAm-containing nanofibers occurs when the temperature is raised between 35ºC and up to above 100º eventually. The remarkably well spinnability while maintaining the thermo responsiveness of the coaxial fibers was achieved by the use of appropriate ratio of EC/PNIPAAm. However, comparing to pure PNIPAAm nanofibers and the blended nanofibers, the water contact angle of coaxial nanofibers reaches the highest contact at about 135º. This result indicates that coaxial nanofibers have a better hydrophobic than blended nanofibers in both lower temperature and higher temperatures.

The water contact angle (CA) of nanofibers with drug-loaded (A4, A5, A6) was also identified which have similar change to those of drug-free nanofibers. KET loaded fibers produced a little change for the water contact angle.

(Figure 6)

Fig. 7 shows the proposed mechanism underlying the thermoresponsive properties of the PNIPAAm/EC coaxial nanofibers. When the temperature is below the LCST (32ºC), the hydrophilic C=O and N-H groups in the PNIPAAm chains interact easily with water molecules to form intermolecular hydrogen bonds.[37] In addition, the water droplets can presumably minimize their contact with these and maximize their interactions because the hydrophobic EC molecules wrapped outside. When the temperature is above the LCST, the formation of intra-molecular hydrogen bonds between the C=O and N-H groups in PNIPAAm lead to a collapsed globular conformation of the chains, which makes it very hard for their C=O and N-H groups to interact with water molecules. The EC as sheath also shrank when the core changed their morphology of structure. Therefore, the coaxial fibers are hydrophobic when the temperature above the LCST.

(Figure 7)

3.6 Drug release

(Figure 8)

Fig. 8 displays the *in vitro* release of KET from the KET-loaded nanofibers at 25ºC and 37ºC in PBS at pH 7.4. At 25 ºC, the amount of KET release from A4 (PNIPAAm) is the highest, followed by A5 (PNIPAAm/EC) and A6 (coaxial nanofibers) is the lowest. The different drug release profiles may be explained by their different surface wettability. Hydrophilic carrier tends to be faster than hydrophobic material in drug release.[38] When the temperature is at 25ºC, the pure PNIPAAm fibers show high hydrophilic properties whereas the blended and coaxial fibers exhibit hydrophobic properties. This is due to the presence of the EC in these fibers. The coaxial fibers have lowest hydrophilic properties because the EC is the sheath. At 37ºC, there is a burst release of roughly 40% of the loaded drug in the first 5 h of the dissolution experiment, while A6 shows slower, sustained, release over *ca.* 55 h. A6 releases KET the slowest under these conditions, followed by A5 and A4.

According to previous work in our group, the drug release profile from the EC fibers is very similar at the two temperatures investigated. EC is not a thermoresponsive material, but it can be used as a sustained-release drug delivery vehicle.[30] As the result of the particular temperature sensitive properties of PNIPAAm, the drug release faster and much greater at 25ºC than at 37ºC. However, comparing A5 and A6, the latter is more effective for drug release slowly and constantly. These effects are reduced for A6 samples, indicating that the coaxial nanofibers combine the thermoresponsive and hydrophobicity of the two polymers, possessing both thermosensitive and sustained release properties. Here is the schematic illustration of the core-sheath nanofibers with PNIPAAm and EC (Fig. 7). When the temperature rises, the sheath nanofibers tighten up along with core nanofibers shrank. While the temperature drops, the coaxial nanofibers restore the original state.

3.7 Cell experiments

The results of MTT cell viability measurements on the all fibers are given in Fig. 9. The number of cells increased from day 1 to day 5. The cell viability of KET-free and KET-loaded fibers was similar for samples made from the same materials. MTT assay

did not show significant differences in cell proliferation after 1 day and 3 days. However, there was an increase in cell growth with A3 and A6 after 5 days, compared to the pure PNIPAAm nanofibers and the blended nanofibers. From these results, the prepared nanofibers had been proved to be non-toxic and suitable for cell growth.

(Figure 9)

4. Conclusion

Core-sheath nanofibers with thermoresponsive poly-(N-isopropylacrylamide) (PNIPAAm) as the core and hydrophobic ethyl cellulose (EC) as the sheath were successfully prepared by coaxial spinneret electrospinning. Ketoprofen (KET) was selected as a model drug, and analogous medicated fibers also generated. Compeered to the blended fibers, the coaxial fibers have similar regular morphologies in drug sustained release and biocompatibility. X-ray diffraction data demonstrate that KET exists in the amorphous physical form in the drug-loaded fibers. There are significant intermolecular interactions between KET and the polymers, as evidenced by IR spectroscopy. The water contact angle of nanofibers with PNIPAAm changes abruptly upon heating, when the temperature is increased through the lower critical solution temperature. KET was released at 25ºC with a much faster rate than at 37ºC as a result of this change in properties. The coaxial fibers show very clear temperature-sensitive drug delivery with sustained release than the blended fibers. They also proved to be non-toxic and suitable for cell growth. Thus, the coaxial nanofibers have both thermosensitive and sustained release properties, which could be good thermoresponsive carriers for the sustained release, especially for poorly water soluble drugs and are potential biocompatible nanofibers in biomedical field. The system reported here would be expected to prevent the undesirable side effects that may sometimes arise from non-localized drug release. ~~And the system will be percutaneous administration in humans in a local part.~~

5. Acknowledgements

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6. References

[1] Yang T, Yao Y, Lin Y, et al. Electrospinning of polyacrylonitrile fibers from ionic liquid solution. *Appl Phys A*, 2010, 98(3):517-523.

[2] Jin W J, Jeon H J, Kim J H, et al. A study on the preparation of poly(vinyl alcohol) nanofibers containing silver nanoparticles. *Synth Met*, 2007, 157(10–12):454-459.

[3] Min B M, Lee G, Kim S H, et al. Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts in vitro. *Biomaterials*, 2004, 25(7–8):1289-1297.

[4] Li X, Qian Q, Zheng W, et al. Preparation and Characteristics of LaOCl Nanotubes by Coaxial Electrospinning. *Mater Lett*, 2012, 80(8):43-45.

[5] Wang B, Zhang P P, Williams G R, et al. A simple route to form magnetic chitosan nanoparticles from coaxial-electrospun composite nanofibers. *J Mater Sci*, 2013, 48(11):3991-3998.

[6] Zander N E. Hierarchically Structured Electrospun Fibers. *Polymers*, 2013, 5(1):19-44.

[7] Fu Y, Guan J, Guo S, et al. Human urine-derived stem cells in combination with polycaprolactone/gelatin nanofibrous membranes enhance wound healing by promoting angiogenesis. *J Transl Med*, 2013, 12(1):1-14.

[8] Christina K, Alessandra A, Alessandra A, et al. Fabrication, functionalization, and application of electrospun biopolymer nanofibers. *Crit Rev Food Sci Nutr*, 2008, 48(8):775-797.

[9] Hedges A R. The Application of Soft-Calender. *Chem Rev*, 1998, 98(98):2035-2044.

[10] Qiu S, Liu L, Wang B, et al. Facile Synthesis of Carbazole-Containing Semiladder Polyphenylenes for Pure-Blue Electroluminescence. *Macromolecules*, 2005, 38(16):6782-6788.

[11] Ying L, Kang E T, Neoh K G, et al. Drug permeation through temperature-sensitive membranes prepared from poly(vinylidene fluoride) with grafted poly(N -isopropylacrylamide) chains. *J Membr Sci*, 2004, 243(s 1–2):253-262.

[12] Ying L, Wang P, And E T K, et al. Synthesis and Characterization of Poly(acrylic acid)-graft-poly(vinylidene fluoride) Copolymers and pH-Sensitive Membranes. *Macromolecules*, 2001, 35(3):673-679.

[13] Maxim Orlov , Ihor Tokarev , Andreas Scholl, et al. pH-Responsive Thin Film Membranes from Poly(2-vinylpyridine): Water Vapor-Induced Formation of a Microporous Structure. *Macromolecules*, 2007, 40(40):2086-2091.

[14] Fu-Jian X, En-Tang K, Koon-Gee N. pH- and temperature-responsive hydrogels from crosslinked triblock copolymers prepared via consecutive atom transfer radical polymerizations. *Biomaterials*, 2006, 27(14):2787-2797.

[15] Xian-Zheng Z, Patti J L, Chih-Chang C. Fabrication and characterization of a smart drug delivery system: microsphere in hydrogel. *Biomaterials*, 2005, 26(16):3299-3309.

[16] Huber D L, Manginell R P, Samara M A, et al. Programmed adsorption and release of proteins in a microfluidic device. *Sci*, 2003, 301(5631):352-354.

[17] Schild H G. Schild, H. G. Poly (N-Isopropylacrylamide) - experiment, theory and application. *Prog. Polym. Sci.* 1992, 17(2):163-249.
[18] Huang S R, Lin K F, Lee C F, et al. Synthesis and properties of thermoresponsive magnetic polymer composites and their electrospun nanofibers. *J Polym Sci Part A Polym Chem*, 2014, 52(6):848-856.
[19] Rockwood D N, Chase D B, Akins R E, et al. Characterization of electrospun poly(N-isopropyl acrylamide) fibers. *Polymer*, 2008, 49(18):4025-4032.
[20] Kumoda M, Yukikazu Takeoka A, Watanabe M. Template Synthesis of Poly(N-isopropylacrylamide) Minigels Using Interconnecting Macroporous Polystyrene. *Langmuir*, 2002, 19(3):525-528.
[21] Min S, Chao P, Li J, et al. Blends of TiO 2 nanoparticles and poly (N -isopropylacrylamide)- co -polystyrene nanofibers as a means to promote the biorecognition of an anticancer drug. *Talanta*, 2008, 75(4):1035-1040.
[22] Yamato M, Akiyama Y, Kobayashi J, et al. Temperature-responsive cell culture surfaces for regenerative medicine with cell sheet engineering. *Prog Polym Sci*, 2007, 32(8):1123-1133.
[23] París R, Quijada-Garrido I. Swelling and hydrolytic degradation behaviour of pH-responsive hydrogels of poly[(N -isopropylacrylamide)- co -(methacrylic acid)] crosslinked by biodegradable polycaprolactone chains. *Polym Int*, 2009, 58(4):362–367.
[24] Lee W F, Cheng T S. Studies on preparation and properties of porous biodegradable poly(NIPAAm) hydrogels. *J Appl Polym Sci*, 2008, 109(3):1982–1992.
[25] Rockwood D N, Chase D B, Akins R E, et al. Characterization of electrospun poly(N-isopropyl acrylamide) fibers. *Polymer*, 2008, 49(18):4025-4032.
[26] Wang J, Sutti A, Wang X, et al. Fast responsive and morphologically robust thermo-responsive hydrogel nanofibres from poly(N-isopropylacrylamide) and POSS crosslinker. *Soft Matter*, 2011, 7(9):4364-4369..
[27] Chen M, Dong M, Havelund R, et al. Thermo-Responsive Core−Sheath Electrospun Nanofibers from Poly (N-isopropylacrylamide)/Polycaprolactone Blends. *Chem Mater*, 2010, 22(14):4214-4221.
[28]Gu S Y, Wang Z M, Li J B, et al. Switchable Wettability of Thermo-Responsive Biocompatible Nanofibrous Films Created by Electrospinning. *Macromol Mater & Eng*, 2010, 295(295):32-36.
[29] Um-I-Zahra S, Shen X X, Li H, et al. Study of sustained release drug-loaded nanofibers of cellulose acetate and ethyl cellulose polymer blends prepared by electrospinning and their in-vitro drug release profiles. *J Polym Res*, 2014, 21(12):1-12.
[30] Hu J, Li H Y, Williams G R, et al. Electrospun Poly(N-isopropylacrylamide)/Ethyl Cellulose Nanofibers as Thermoresponsive Drug Delivery Systems. *J Pharm Sci*, 2016. 105(3):1104-1112.
[31] Kulkarni P K, Dixit M, Kumar Y S, et al. Preparation and evaluation of Ketoprofen beads by melt solidification technique. *Der Pharmacia Sinica*, 2010, 1(2):31-43.
[32] Gauri N, Aditi L, Shikhaa A, et al. Solubility enhancement of a poorly aqueous

soluble drug ketoprofen using solid dispersion technique. *Der Pharmacia Sinica*, 2011.
[33] Rockwood D N, Chase D B, Akins R E, et al. Characterization of electrospun poly(N-isopropyl acrylamide) fibers. *Polymer*, 2008, 49(18):4025-4032.
[34]Lopez-Sanchez J A, Cornejo-Bravo J M, Luque P A, et al. Synthsis and comparative study of N-isopropylamide (NIPAAm) hydrogel and N-isopropylamide-methyl-methacrylate (NIPAAm-MMA) gel. *Dig J Nanomater Bios*, 2015, 10(1):161-167.
[35] Kang H, Liu W, He B, et al. Synthesis of amphiphilic ethyl cellulose grafting poly(acrylic acid) copolymers and their self-assembly morphologies in water. *Polymer*, 2006, 47(23):7927–7934.
[36] Jones D M, Smith J R, Huck W T S, et al. Variable Adhesion of Micropatterned Thermoresponsive Polymer Brushes: AFM Investigations of Poly(N -isopropylacrylamide) Brushes Prepared by Surface-Initiated Polymerizations. *Adv Mater*, 2002, 14(16):1130–1134.
[37] Wang N, Zhao Y, Jiang L. Low‐Cost, Thermoresponsive Wettability of Surfaces: Poly(N‐isopropylacrylamide)/Polystyrene Composite Films Prepared by Electrospinning. *Macromol Rapid Commun*, 2008, 29(29):485-489.
[38] Yoshida R, Sakai K, Okano T, et al. A New Model for Zero-Order Drug Release I. Hydrophobic Drug Release from Hydrophilic Polymeric Matrices. *Polym J*, 1991, 23(9):1111-1121.

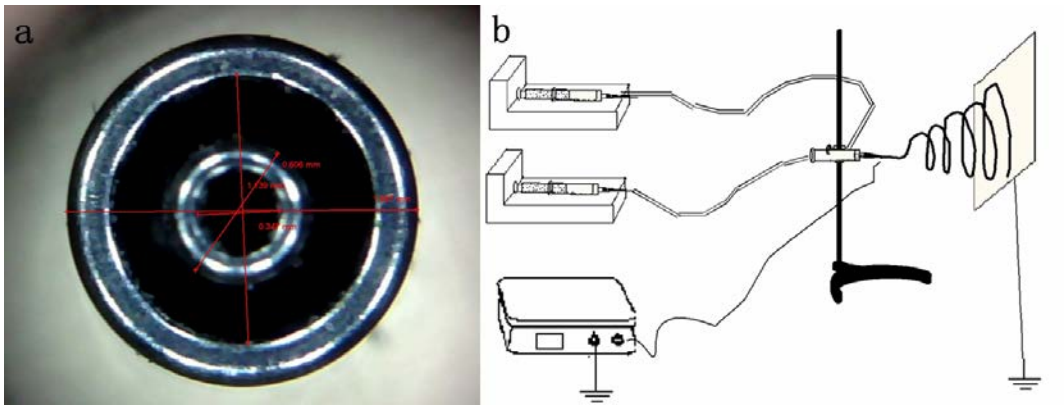

Fig. 1 Schematic diagram of the equipment for the electrospinning process of coaxial nanofibers.

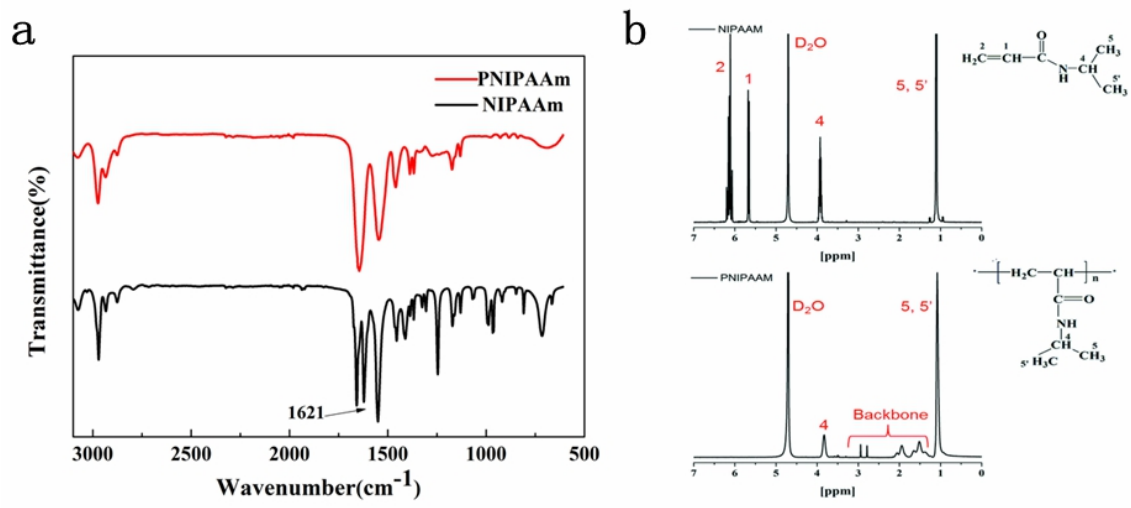

Fig. 2. FTIR spectra of (a) NIPAAm and PNIPAAm and (b) 1-H NMR spectra of NIPAAm and PNIPAAm.

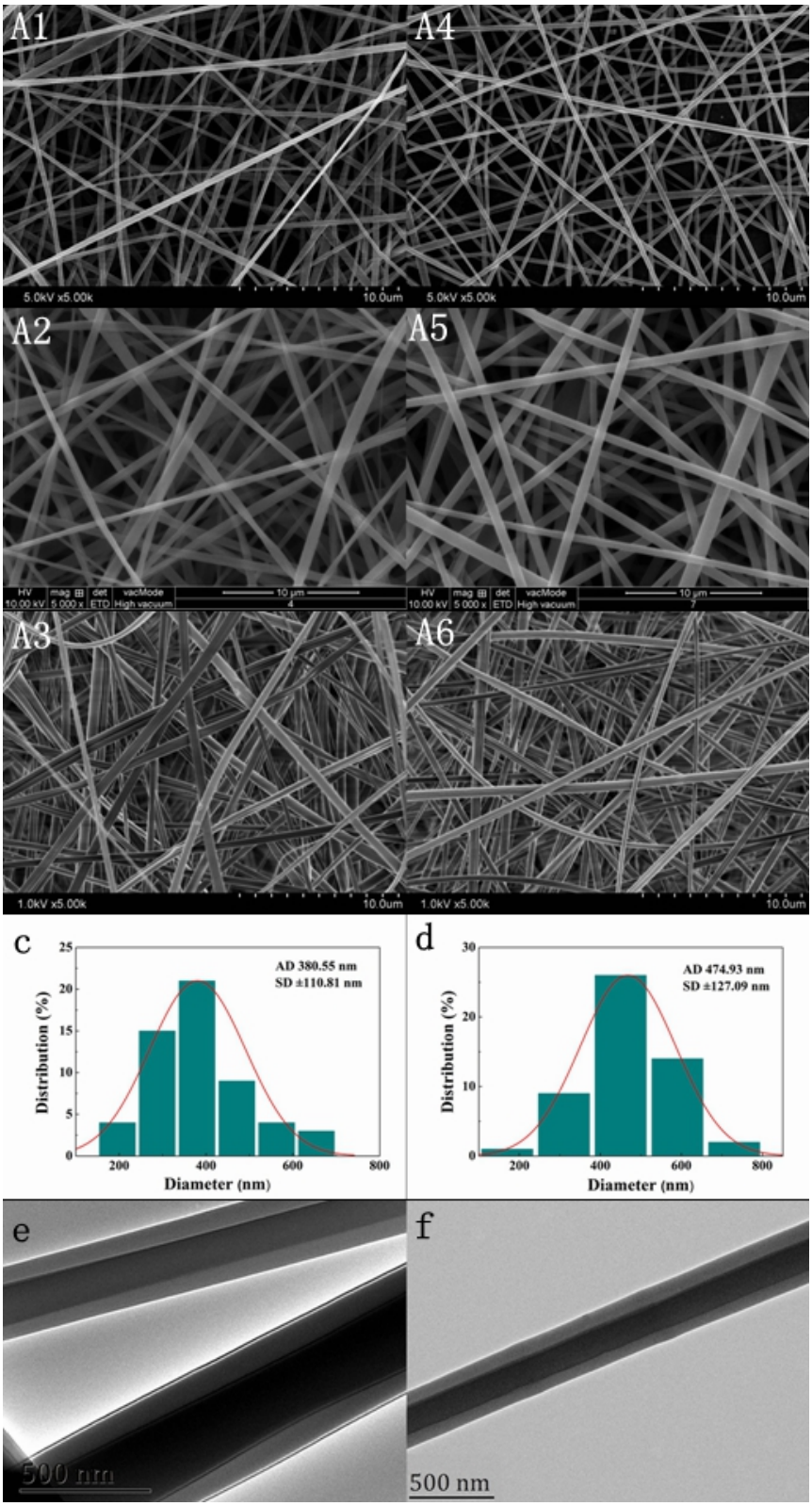

Fig. 3 SEM images of the all nanofibers: pure PNIPAAm nanofibers (A1), blend nanofibers (A2), coaxial nanofibers (A3), and pure PNIPAAm nanofibers with KET (A4), blend nanofibers with KET (A5), coaxial nanofibers with KET (A6); diameters of coaxial nanofibers (c) and coaxial nanofibers with drug loaded (d); TEM images of coaxial nanofibers with

drug-free (e) and drug-loaded (f).

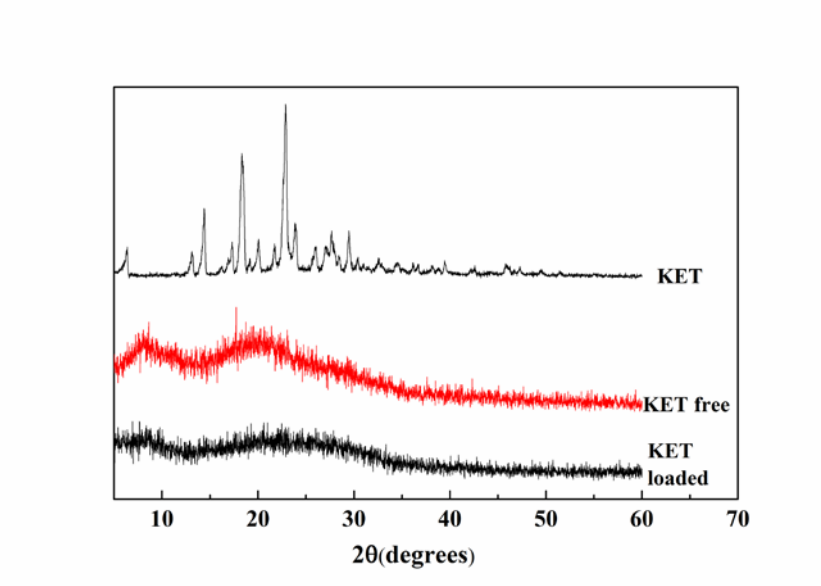

Fig. 4. XRD patterns of KET and the coaxial nanofibers: coaxial nanofibers with KET free and loaded.

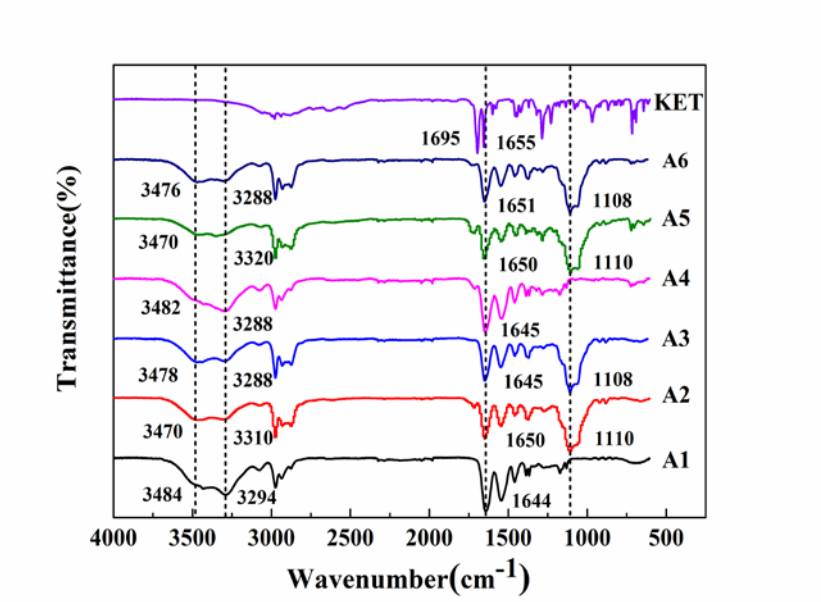

Fig. 5. FTIR spectra of KET and all the fibers: PNIPAAm nanofibers (A1); PNIPAAm/EC blended nanofibers (A2); coaxial nanofibers (A3); PNIPAAm/KET nanofibers (A4); PNIPAAm/EC/KET blended nanofibers (A5) and coaxial nanofibers with drug loaded (A6).

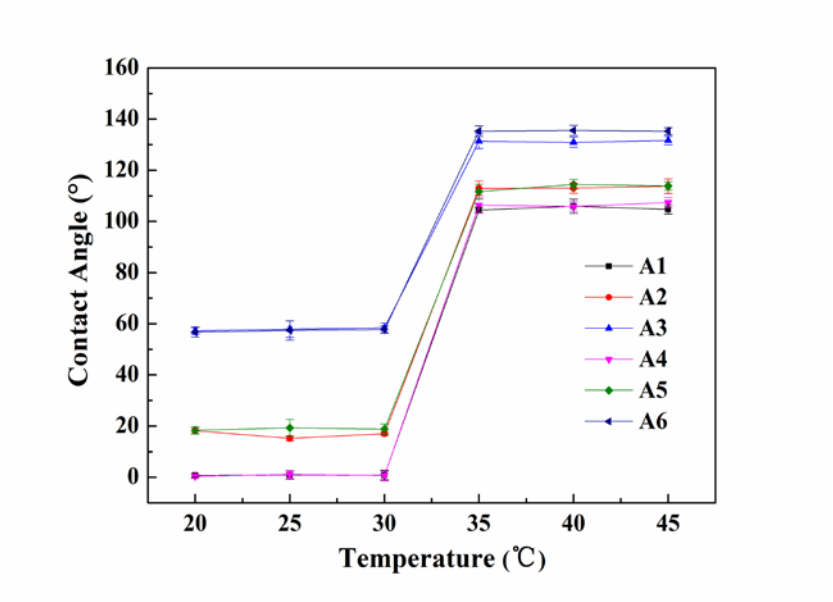

Fig. 6. Contact angles of nanofibers at different temperatures. PNIPAAm nanofibers (A1); PNIPAAm/EC blended nanofibers (A2); coaxial nanofibers (A3); PNIPAAm/KET nanofibers (A4); PNIPAAm/EC/KET blended nanofibers (A5) and coaxial nanofibers with drug loaded (A6).

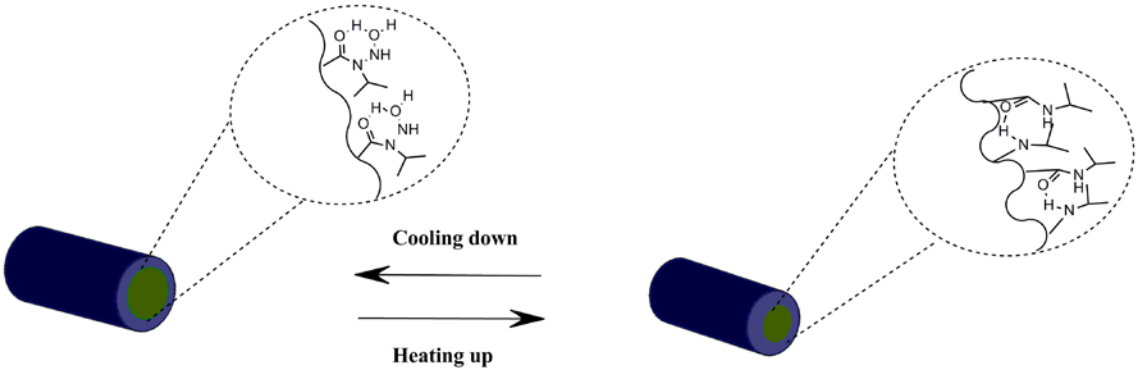

Fig. 7. A proposed mechanism underlying the thermoresponsive properties of the PNIPAAm/EC coaxial fibers.

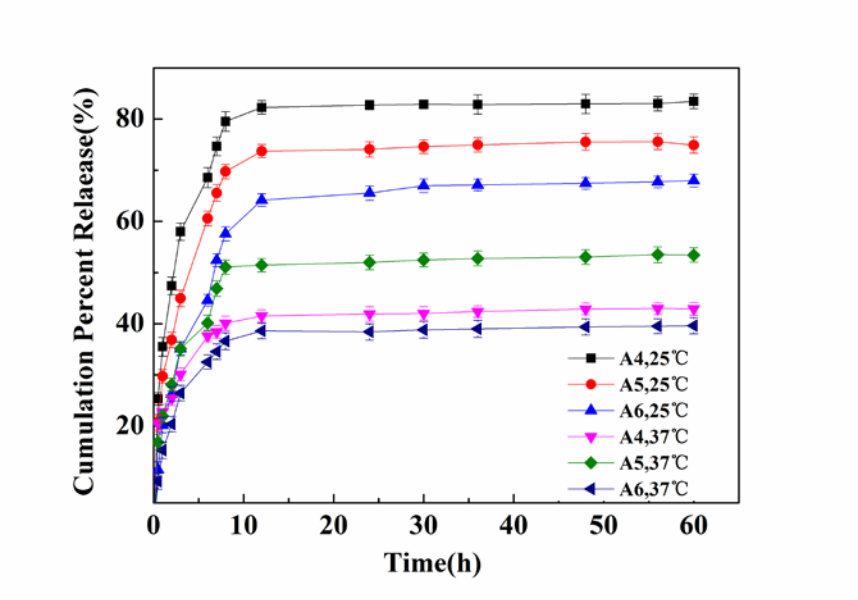

Fig. 8. *In vitro* KET release profiles from the electrospun fibers. PNIPAAm/KET nanofibers (A4); PNIPAAm/EC/KET blended nanofibers (A5) and coaxial nanofibers with drug loaded (A6).

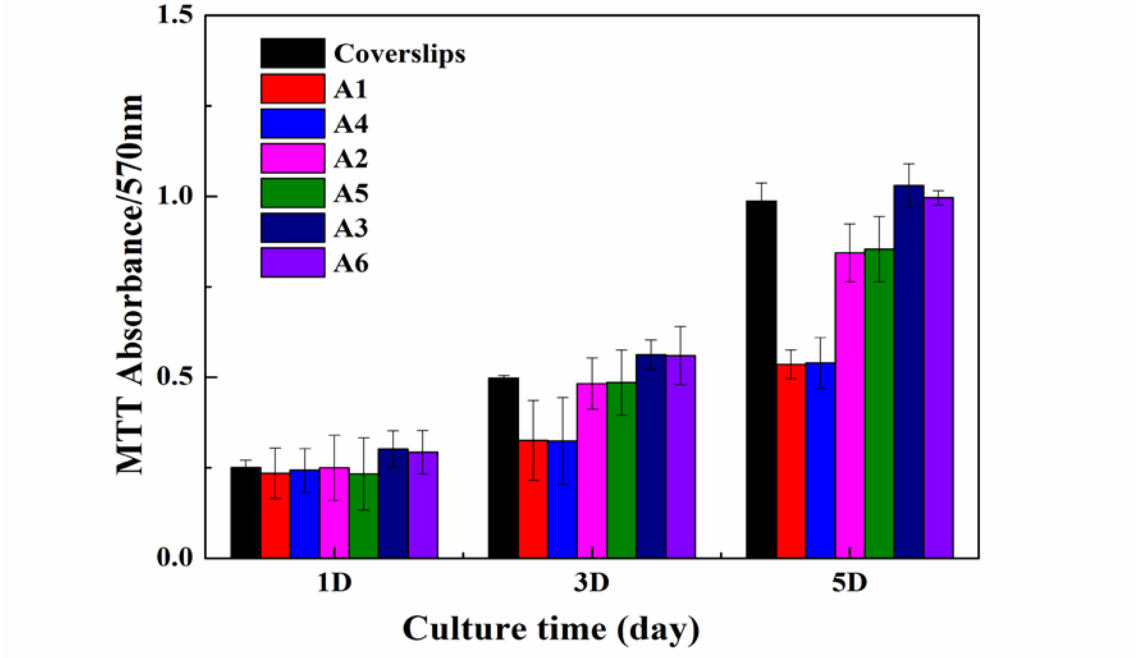

Fig. 9. Cell viability of L929 cells grown on blank coverslips and those coated with the fibers. PNIPAAm nanofibers (A1); PNIPAAm/EC blended nanofibers (A2); coaxial nanofibers (A3); PNIPAAm/KET nanofibers (A4); PNIPAAm/EC/KET blended nanofibers (A5) and coaxial nanofibers with drug loaded (A6).