Materials Science and Engineering C 72 (2017) 293–300

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Temperature-responsive PLLA/PNIPAM nanofibers for switchable release

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ARTICLE INFO

Article history:
Received 6 September 2016
Accepted 7 November 2016
Available online 9 November 2016

Keywords:
Responsive release
Polymer blends
Electrospinning
Nanofibers
Stimuli-responsive
Switchable wettability
Antibacterial

ABSTRACT

Smart antimicrobial materials with on-demand drug release are highly desired for biomedical applications. Herein, we report about temperature-responsive poly(N-isopropylacrylamide) (PNIPAM) nanospheres doped with crystal violet (CV) and incorporated into the poly-L-lactide (PLLA) nanofibers. The nanofibers were prepared by electrospinning, using different initial polymers ratios. The morphology of the nanofibers and polymers distribution in the nanofibers were characterized by scanning electron microscopy (SEM) and atomic force microscopy (AFM). The interaction between PNIPAM and PLLA in the nanofibers was studied by Fourier transform infrared spectroscopy (FTIR) and its effect on the PNIPAM phase transition was also investigated. It was shown that by the changing of the environmental temperature across the lower critical solution temperature (LCST) of PNIPAM, the switchable wettability and controlled CV release can be achieved. The temperature-dependent release kinetics of CV from polymer nanofibers was investigated by ultraviolet-visible spectroscopy (UV–Vis). The temperature-responsive release of antibacterial CV was also tested for triggering of antibacterial activity, which was examined on *Staphylococcus epidermidis (S. epidermidis)* and *Escherichia coli* (*E. coli*). Thus, the proposed material is promising value for controllable drug-release.

1. Introduction

The development of a new class of materials that provides on-demand switchable drug release represents one of the most actual challenges in the biomedical field [1,2]. Such materials provide a possibility to achieve the necessary drug concentration in the right place at the right time, enable a significant decrease of exposure time and prevent premature release, reducing the risk of developing bacterial resistance [3]. Most of the recent studies in the area of smart materials with switchable drug release have been dedicated to the creation of antibacterial materials based on the so-called stimuli-responsive polymers [4]. In general, stimuli-responsive, or smart polymers can undergo the basic physico-chemical changes in response to various external stimulus: temperature, light, pH, appearance of enzymes or ions, triggering by magnetic or electric fields [5–11].

One of the most-studied responsive polymers is poly(N-isopropylacrylamide) (PNIPAM) which has a lower critical solution temperature (LCST) of 32 °C. Below LCST PNIPAM is soluble in aqueous solution, but above LCST the intramolecular hydrogen bonding dominate and the polymer becomes insoluble. This property makes PNIPAM an attractive material for the temperature-controlled drug release [12]. The further implementation of PNIPAM in the medical field requires the extension of the available temperature range (i.e. shift of the polymer phase transition) and acceleration or deceleration of drug release kinetic [4]. As the drug release speed is concerned, the preparation of different PNIPAM-based nano-/microstructures seems to be promising [13].

In the last decade, PNIPAM is actively used for electrospinning of micro and nanofibers [14]. Such structures have a huge surface-to-volume ratio that makes them an attractive material as a carrier for antibacterial and anticancer drugs [15–17] and provides more rapid response and complete drug release. Moreover, the spatial confinement of PNIPAM and its physical interaction with another component in the polymer blend can help to tune the temperature of polymer phase transition [18–20].

The LCST of PNIPAM can be tuned by copolymerization of NIPAm monomer or by a blending with other polymers [21,22]. For example, Gao et al. [23] reported about poly(N-isopropylacrylamide)-*co*-poly(acrylic acid) derivative and demonstrate their potential for controlled release of insulin. Another possibility of PNIPAM is realized through PNIPAM grafting on a supported surface [24,25]. A simpler PNIPAM blending with the polymers, containing polar group able to interact with PNIPAM, is also a perspective way to shift the polymer phase transition [26,27].

As a potential drug delivery system, the combination of nanosized PNIPAM with the polymers blend was used [28–30]. The use of blends brings some benefits. Yarin et al. prepared and characterized nanofibers composed of poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA) and PNIPAM nanogel. This "nano-resins" do not show global shrinking/swelling, but demonstrate thermo-responsive release [31]. Song et al.

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http://dx.doi.org/10.1016/j.msec.2016.11.028
0928-4931/

[32] used PNIPAM/poly(ethylene oxide) blend as a potential drug delivery system and PNIPAM/PMMA nanofibers for reversible adsorption of BSA. Ganesh et al. [33] reported about cellulose acetate/PNIPAM nanofibrous membranes and demonstrated its switchable wettability behavior. So, the approach based on responsive polymers is extremely interesting in the various fields of research, with a main focus on the medical application. Further development of these attractive researches lies in the expansion of dynamics and range of materials response.

In this work, we report about thermo-responsive polymer nanofibers prepared by electrospinning from PLLA/PNIPAM nanospheres blend, containing crystal violet (CV) as a model drug. Electrospinning provides the unique feature of one-step nanofibers fabrication from polymer blends. We investigate thermo-responsive properties of PLLA/PNIPAM nanofibers with different polymer ratios. The release of antibacterial CV for triggering of antibacterial activity against *Staphylococcus epidermidis* and *Escherichia coli* strains was also tested.

2. Experimental

2.1. Materials

Poly-L-lactic acid (PLLA) was purchased from Goodfellow, Ltd., UK, N-Isopropylacrylamide (NIPAm), N,N′-Methylenebisacrylamide (MBA), potassium persulfate (KPS), sodium dodecyl sulfate (SDS), crystal violet (tris(p-dimethylaminophenyl)methyl chloride, CV) were obtained from Sigma Aldrich and used without further purification. Chloroform (99.5%) and ethanol (99%) were purchased from PENTA. Mueller-Hinton agar (MHA) were prepared as described by producer (Oxoid, CM0337) and sterilized in the autoclave.

2.2. Preparation of PLLA/PNIPAM nanofibers

2.2.1. PNIPAM nanospheres synthesis

First, 50 ml of pristine water was placed in round-bottom flask and bubbled with argon. Then 1.13 g NIPAm, *N,N′*-Methylenebisacrylamide (MBA) 0.0616 g and 0.027 g of KPS were added and mixed by a magnetic bar until total dissolution. Then, SDS was added to the mixture and after its dissolution, the mixture was heated up to 60 °C. The polymerization was performed for 60 min under argon and s terminated by immersing in the ice-water bath. After the polymerization termination the mixture was placed in dialysis bags with cutoff 3000–5000. The polymer was dialyzed for 1 week with refreshing the water twice a day. Next, the polymer was lyophilized and characterized by FTIR and TEM.

2.2.2. Solution preparation

PLLA/PNIPAM and PNIPAM were dissolved in the mixed solvent of chloroform and ethanol (2:1 *v*/v) to obtain 3% (*w*/w) 1:1, 3:1, 9:1 solutions and then, appropriate amount of crystal violet (CV) was added to obtain 1%. The solutions were placed in the plastic syringe with 25 G needle.

2.2.3. Electrospinning

For electrospinning the vertical set up containing a syringe pump (NE-300, New Era Pump Systems, Inc.) was used (Fig. 1). The nanofibers were prepared with a 1 ml h^{-1} feeding rate, 80 mm tip-to-collector distance and the voltage 18 kV. The metal needle tip was clamped to the positive electrode of high voltage power supply (homemade). The PLLA/PNIPAM nanofibers containing CV were collected for 5 min on the aluminum foil. For the antibacterial tests the glass substrates were previously sputtered with 50 nm Au layer to prevent the formation of insulation zones during electrospinning. In order to remove any residual solvent, the nanofibers were dried overnight under vacuum.

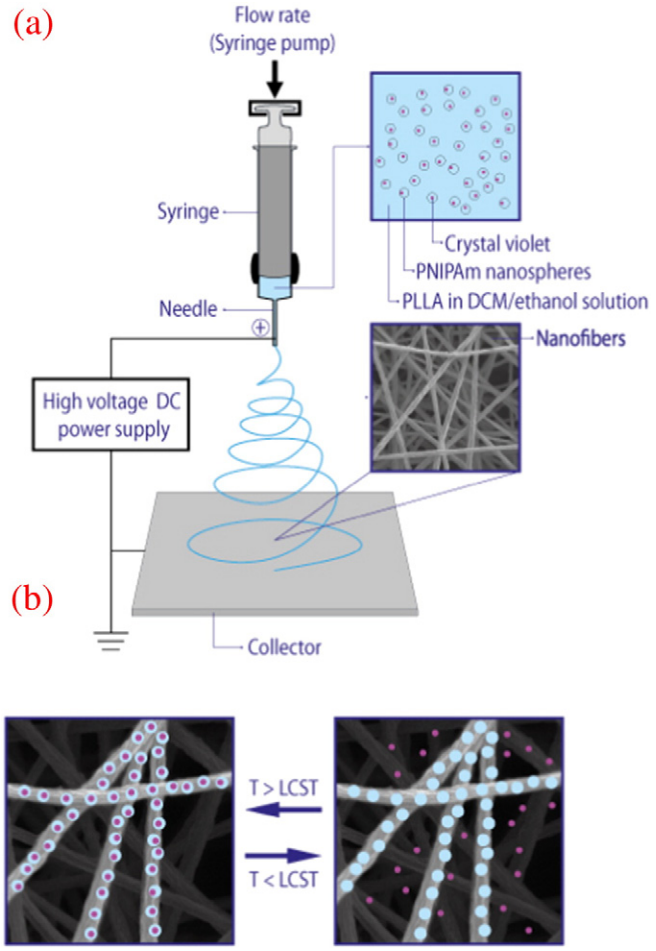

Fig. 1. (a) – Schematic representation of electrospinning procedure and crystal violet (CV) encapsulation; (b) – Schematic representation of materials phase transition and CV release from PLLA/PNIPAM nanofibers.

2.3. Samples characterization

PNIPAM nanospheres were observed by transmission electron microscopy (TEM) (JEOL JEM-1010 transmission electron microscope, with SIS MegaView III digital camera). For TEM observation the nanospheres were collected on carbon-coated copper grids. The morphology of the nanofibers was observed by scanning electron microscopy (SEM) (LYRA3 TESCAN). For SEM observation the samples were preliminary sputtered with 50 nm platinum layer. The distribution of the fibers was determined using ImageJ software. For the characterization of sample surfaces and polymers distribution mapping, the peak force AFM was applied. Surface mapping was performed with Icon (Bruker) setup on the areas of 400 × 400 nm^2. Refraction spectra of thin nanofibers layer deposited onto Si substrate were determined in-situ under cooling from 40 °C to 25 °C in 250–750 nm spectral range using refractometer Avaspec 2048. After each set of measurements the wavelength, at which the change of refraction intensity due to polymer phase transition is maximal, was determined (450 nm) and used as a characteristic wavelength. UV–Vis spectra were measured by Lambda 25 Spectrometer (Perkin-Elmer) in 300–1100 nm wavelength range. The interaction between PNIPAM and PLLA in the nanofibers was confirmed by Fourier transform infrared (FTIR) spectroscopy using total reflectance mode (ATR) on Nicolet 6700 spectrometer (Thermo Scientific, France). The approval testing of the switching of surface wettability was realized by automatic contact angle measurement (SEE System, Advex Instruments, Czech Republic). Water drops of the constant volume 2 μl were die-