Materials Science and Engineering C 79 (2017) 245–254

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Poly(*N*-isopropylacrylamide)/poly(L-lactic acid-*co*-ε-caprolactone) fibers loaded with ciprofloxacin as wound dressing materials

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ARTICLE INFO

Article history:
Received 16 November 2016
Received in revised form 5 April 2017
Accepted 11 April 2017
Available online 5 May 2017

Keywords:
Wound dressing
Electrospinning
Thermosensitive
PNIPAAm
Antibacterial

ABSTRACT

In this work, we aimed to develop new materials to reduce the secondary injuries which can be imparted when replacing wound dressings. Electrospun fibers based on the thermoresponsive polymer poly(*N*-isopropylacrylamide) (PNIPAAm), poly(L-lactic acid-*co*-ε-caprolactone) (PLCL), and the antibiotic ciprofloxacin (CIF) were prepared. The water contact angle of fibers made from a blend of PNIPAAm and PLCL changed dramatically when the temperature was increased above 32 °C. Sustained release of CIF from the formulations was observed over >200 h. Moreover, L929 fibroblasts could proliferate on the fibers, indicating their biocompatibility. The CIF-loaded fibers were found to have potent antibacterial activity against *E. coli* and *S. aureus*. *In vivo* tests on rats indicated that CIF-loaded thermosensitive fibers have enhanced healing performance compared to CIF-loaded PLCL fibers or a commercial gauze. Electrospun PNIPAAm/PLCL fibers loaded with CIF thus have great promise in the development of new wound dressing materials.

1. Introduction

Wound healing is a complicated biological process involving numerous physiological factors. To accelerate the healing process, wound dressings have been used since ancient times [1]. Modern dressings must both provide basic protection for the wound site and also accelerate the healing process [2,3]. Various materials have been reported in the literature in attempts to achieve the latter, including foams, films, hydrocolloids, hydrogels, and hydrofibers (*inter alia*) [4–8]. Electrospun fibers have attracted much attention in this regard, and have a number of potential advantages as wound dressing materials.

Electrospinning is a simple and effective method for producing fibers with diameters ranging from tens of nanometers to micrometers. The materials fabricated by this route not only have high porosity and surface area-to-volume ratios (with the possibilities of tuning both), but also resemble the morphological structure of the extra-cellular matrix and generally have good biocompatibility [9–11]. As a result of these characteristics they have hemostatic properties, and are absorbable and semi-permeable, giving great promise as wound dressing materials [12]. Furthermore, various functional ingredients can easily be incorporated into fibers *via* electrospinning [1]. This could yield multi-functional wound healing materials.

To date, a number of polymers have been used to fabricate electrospun wound dressings, including gelatin, collagen, chitosan, poly(vinyl alcohol), poly(ε-caprolactone), and poly(L-lactic acid) [3,13–16]. Of particular interest is poly(L-lactic acid-*co*-ε-caprolactone) (PLCL),a copolymer of L-lactic acid and ε-caprolactone which has outstanding mechanical properties, biocompatibility and degradability [17]. The use of electrospun PLCL scaffolds has been widely investigated in tissue regeneration [18,19], but has attracted much less attention in the context of wound dressing materials.

Beyond these simple systems, more complex stimuli-responsive polymers have been shown to have great promise in biomaterials, drug delivery systems and sensors [20–25]. They exhibit a change in configuration, dimensions, or physicochemical properties after exposure to an external stimulus, such as changes in temperature, pH, electromagnetic field, or light [26–28]. Poly(*N*-isopropylacrylamide) (PNIPAAm) is a typical temperature-sensitive polymer. In aqueous solution it undergoes a sharp phase transition from linear to globular at a lower critical solution temperature (LCST) of 32 °C [29–31]. When the temperature is raised through the LCST, PNIPAAm changes from being hydrophilic to hydrophobic [32–34]. It has been intensively studied for potential applications in biomedicine [35–38], and has also been electrospun successfully [33,39,40]. Moreover, it has been found that thermosensitive materials like PNIPAAm can effectively control cell adhesion and detachment through variation in the temperature [41]. The strength of interactions between the polymer and cells is generally increased above the LCST, and thus if a polymer has an LCST a little

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http://dx.doi.org/10.1016/j.msec.2017.04.058
0928-4931/

below the human body temperature a low-temperature treatment can be applied to reduce attractions between the polymer and cells [42]. This could in turn reduce secondary injuries caused when a dressing is removed from a wound.

Several researchers have reported thermosensitive wound dressings generated from PNIPAAm [43–45]. For example, systems based on PNIPAAm, polyurethane and chitosan have been proven to exhibit good biocompatibility, antibacterial ability and water vapor transmission [46]. In other work, Yang et al. found that polymer membranes synthesized from PNIPAAm, methyl methacrylate, and 2-hydroxyethyl methacrylate have low cytotoxicity, promising thermosensitivity and good cell detachment properties [43], with 90% of L929 fibroblast cells being detached at 15 °C. However, most of the reported thermosensitive dressings based on PNIPAAm were not fabricated by electrospinning, and we hypothesized that preparing similar systems in the form of electrospun fibers would result in a number of advantageous properties.

The work described in this paper forms part of a programme of research aiming to prepare wound dressings from electrospun fibers. We focus on blend materials containing both a thermosensitive polymer and a second material to provide the required mechanical properties. Previously, we have explored electrospun fibers based on poly(di(ethylene glycol) methyl ether methacrylate) and PLCL, and found these to be promising in reducing secondary injuries [47]. However, there remains the need to systematically explore a wide range of polymers in order to identify the optimum system for use in the clinic, and this work builds on our previous results to investigate an alternative thermosensitive polymer. Here, we thus blended PNIPAAm and PLCL to fabricate composite fibers *via* electrospinning. Ciprofloxacin (CIF), a fluoroquinolone antibiotic, was also incorporated to provide antibacterial properties. *In vitro* and *in vivo* investigations of the CIF-loaded PNIPAAm/PLCL wound dressing materials were undertaken. This is the first time PNIPAAm/PLCL fibers have been electrospun and explored for their potential in wound dressings.

2. Experimental details

2.1. Materials

N-isopropylacrylamide (NIPAAm) was purchased from Japan TCI (Japan). Ciprofloxacin (CIF, purity ≥ 98%), phosphate-buffered saline (PBS), sodium azide, penicillin, trypsin and thiazolyl blue (MTT) were procured from Sigma-Aldrich Ltd. (USA). Azobisisobutyronitrile (AIBN), anhydrous ethanol, acetone, formaldehyde and n-hexane were obtained from the Sinopharm Chemical Reagent Co., Ltd. (China). Poly(L-lactic acid-*co*-ε-caprolactone) (PLCL, 50:50; Mw = 34.5×10^4 g/mol) was provided by Nara Medical University (Japan). 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, 99.5%) came from the Aladdin Industrial Corporation (China). 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 sourced from Jinuo Biological Medicine Technology Ltd. (China). All other chemicals used were analytical grade, and water was doubly distilled before use.

2.2. Polymerization of NIPAAm

PNIPAAm was prepared by free-radical polymerization in accordance with previous work [48]. NIPAAm (5.0 g) and AIBN (25.0 mg) were dissolved in anhydrous ethanol (10 mL) and heated at 70 °C under a positive pressure of N_2. After 7 h, the resultant polymer was precipitated in n-hexane. This crude product was re-precipitated from 50 mL of acetone into 200 mL of n-hexane three times, and the purified material dried for 3 days in a vacuum oven (DZF-6050, Shanghai Laboratory Instrument Work Co. Ltd., China). Successful polymerization was verified by ^{1}H nuclear magnetic resonance (AV-400 instrument, Bruker, Germany) and IR spectroscopy (Nicolet-Nexus 670 FTIR spectrometer, Nicolet Instrument Corporation, USA). Molecular weights (Mw and Mn) and molecular weight distributions were determined by gel permeation chromatography (GPC) measurements on a Waters LS measurement system (Waters, USA) with tetrahydrofuran (THF) as the solvent. The flow rate was 1.0 mL/min, and the column temperature was 35 °C. The molecular weight distribution was calibrated with standard polystyrene samples.

2.3. Preparation of electrospinning solutions

PNIPAAm and PLCL were co-dissolved in HFIP under magnetic stirring for 15 h at room temperature to obtain clear and homogenous solutions. The component ratios of PNIPAAm to PLCL were 1:1 or 1:2 (w/w), and the total concentration of polymer was 10% (w/v). Solutions of PLCL alone (10% w/v in HFIP) were also prepared as controls. The antibiotic CIF was added into the solutions at a drug to polymer ratio of 1:10 (w/w) [49]. Full details of the solutions prepared are listed in Table 1.

2.4. Electrospinning

Each of the electrospinning solutions was placed into a 5 mL plastic syringe fitted with a stainless steel needle (internal diameter 0.5 mm), and the syringe mounted on a syringe pump (KDS100, Cole-Parmer, USA). The solution was expelled from the syringe at a rate of 1.0 mL/h and a high voltage power supply (ZGF-2000, Shanghai Sute Electrical Co. Ltd., China) used to apply a voltage of 14 kV between the needle and a grounded collector (a flat piece of aluminum foil of 12 × 12 cm in size). The distance between the needle tip and the grounded collector was fixed at 10 cm. The relative humidity was *ca.* 40%, and the temperature 25 °C. After electrospinning for 5 h, the products were stored in a vacuum oven at room temperature for 24 h to remove residual solvent.

2.5. Morphological observations

The morphology of the fibers was observed by scanning electron microscopy (SEM; JSM-5600 LV microscope, JEOL, Tokyo, Japan). Samples were first gold sputter-coated for 60 s under argon to make them electrically conductive. They were then imaged using SEM at a voltage of 10 kV. The average fiber diameter for each sample was calculated by measuring approximately 100 fibers in SEM images, using the ImageJ software (National Institutes of Health, USA) [50].

2.6. Further characterization

Characterization was undertaken following the best-practice reported in the literature [1,3,10,17,18,50]. X-ray diffraction (XRD) was performed on a D/Max-BR diffractometer (Rigaku, Japan). The instrument is supplied with Cu Kα radiation (1.5418 Å; 40 kV and 30 mA), and patterns were collected over the 2θ range 5–60°.

Fourier transform infrared (FTIR) spectra were obtained using a Nicolet-Nexus 670 FTIR spectrometer (Nicolet Instrument Corporation, USA) over the range 500–4000 cm^{-1}, at a resolution of 2 cm^{-1}.

Table 1
Details of the electrospinning solutions prepared. The total polymer concentration was 10% w/v in all cases.

Sample	Solution contents	PNIPAAm to PLCL ratio (w/w)	Drug concentration (% w/v)
S1	PNIPAAm/PLCL	1:1	–
S2	PNIPAAm/PLCL	1:2	–
S3	PLCL	–	–
S4	PNIPAAm/PLCL/CIF	1:1	1.0
S5	PNIPAAm/PLCL/CIF	1:2	1.0
S6	PLCL/CIF	–	1.0