Thermoresponsive Poly (N-Isopropylacrylamide)/Polycaprolacton Nanofibrous Scaffolds for Controlled Release of Antibiotics

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Smart antibacterial materials capable of releasing antibiotic drugs upon exposure to external triggers are highly desired for various medical applications. Herein, the fabrication of thermosensitive drug-loaded core–shell nanofibers using the electrospinning technique combined with in situ UV photopolymerization is reported on. The electrospinning method is used for shaping the core structure comprising biodegradable polymer polycaprolactone (PCL). The PCL fibers are coated with the temperature-responsive poly-N-isopropylacrylamide (PNIPAM) via a UV photopolymerization process that allows to precisely control the shell thickness as verified by transmission electron microscope (TEM) analysis. The temperature-dependent switchable wettability of prepared core–shell fibers is investigated and visualized though water contact angle measurements below and above the lower critical solution temperature. Loading of the antibiotic drug doxycycline hyclate (Doxy) in the PCL core nanofibers results in drug-encapsulating fiber meshes that allow diffusion of drug molecules through the PNIPAM shell in a temperature-dependent manner. The antibacterial activity is examined using Gram-negative Escherichia coli (E. coli) as well as Gram-positive Staphylococcus aureus (S. aureus) bacteria. The results demonstrate the high suitability of prepared biocompatible electrospun core–shell PCL/PNIPAM nanofibers as carriers for antibiotic drugs with temperature-sensitive release behavior.

1. Introduction

Electrospinning offers an inexpensive and versatile method for creating nanofibers, meshes, and yarns of biocompatible polymers, both as single-phase and as composite materials, which exhibit promising potential for biomedical applications such as tissue-engineered scaffolds, wound dressing, and drug delivery. Previous studies have shown that nanofiber mats prepared by electrospinning behave very similar to natural extracellular matrices (ECM) and were found to be effective in guiding cell adhesion, growth, and differentiation.1,2 Given its high biocompatibility and biodegradability, polycaprolactone (PCL) constitutes one of the most frequently used polymers in the electrospinning processes and has been approved by the USA Food and Drug Administration Agency (FDA) for use in tissue engineering and drug delivery devices.3,4 For a controlled release of loaded drugs, hybrid structures with stimuli-responsive materials are highly desired. Especially thermoresponsive polymers that exhibit a phase transition upon temperature change in the practically relevant biological window have received increasing attention for drug delivery applications.5,6 Poly(N-isopropylacrylamide) (PNIPAM) represents one of the prominent thermoresponsive polymers with a lower critical solution temperature (LCST) of about 32 °C in aqueous medium,10,11 below which, the hydrophilic C=O and N–H groups in the PNIPAM chain interact readily with water to form intermolecular hydrogen bonding, so that the polymer becomes highly hydrated and swollen.12,13 Above the LCST point, the entropic contributions dominate enthalpic contributions. PNIPAM chains tend to form intramolecular hydrogen bonding between N–H and C=O groups, resulting in collapse and precipitation of the polymer in aqueous solution.14,15 Furthermore, it has been shown that the LCST of PNIPAM can be modulated through the addition of salts,6 solvents,7 or surfactants8 and can approach the human body temperature (37 °C) upon copolymerization with hydrophilic or hydrophobic monomers.9,10 So far, PNIPAM has been successfully used in the fabrication of hydrogels,11 micelles,12 polymeric nanoparticles, and nanoparticles.13,14 Electrospun nanofibers possess several advantages, such as a comparably large surface area, high response rates, and an intrinsically high drug loading capacity.15 To our knowledge, there is no report to date on the fabrication of PNIPAM composite nanofibers using electrospinning process followed by UV-induced photopolymerization for the controlled release of the hydrophilic tetracycline derivative doxycycline hyclate. In this work, a hydrophilic PNIPAM shell was generated.

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by UV photopolymerization using the monomer NIPAM, around a hydrophobic PCL core structure produced by electrospinning technique. Resulting thermoresponsive nanofibrous scaffolds were used as carriers for antibiotic drugs and a temperature-dependent release of the therapeutic was demonstrated.

2. Results and Discussion

2.1. Synthesis and Characterization of Crosslinked Electrospun PCL/PNIPAM Nanofibers

Core–shell PCL/PNIPAM nanofibers were prepared by electrospinning of a PCL solution followed by UV-assisted surface photopolymerization of NIPAM for different durations (0–6 min). The scanning electron microscope (SEM) analysis of the nanofibers revealed a homogeneous morphology of fibers with an average diameter of 275 nm for untreated nanofibers (Figure 1a). With increasing UV irradiation time (2 min, 4 min, and 6 min, Figure 1b-d), the surface roughness of the fibers was found to gradually increase due to progressive degree of crosslinking, evident in the appearance of small grains, which suggested the beginning of NIPAM polymerization on the surface of the PCL core fibers and simultaneously provided an indication for reduced conductivity of the sample. The core–shell structure of as-prepared fibers was additionally investigated by transmission electron microscope (TEM) analysis to study the double-walled structure. The observed strong difference in contrast between core and shell material in the TEM image (Figure 2) supported the successful formation of a composite core–shell structure based on UV photopolymerization of the shell. The photoinitiator (PI) 1-hydroxycyclohexylphenylketon loaded in PCL core fibers initiated the polymerization, and the crosslinked PNIPAM shell was produced in the presence of the crosslinker MBA. After 2 min of UV irradiation, PCL/PNIPAM nanofibers with a core diameter of 295 nm and a shell thickness of 110 nm were observed (Figure 2a). Moreover, an increase in PNIPAM shell thickness to 155 nm was visible after UV exposure time of 4 min (Figure 2b), which indicated that UV exposure time of 2 min was not sufficient to polymerize all NIPAM monomers.

The influence of the UV irradiation time on the fiber diameter has been previously observed, e.g., by Zhang et al., who produced polylactic acid (PLA)/PNIPAM electrospun nanofibers with a defined core–shell structure using UV photopolymerization for 2 min.[19]

In case of a UV exposure time of 6 min (Figure 2c), a 410 nm inhomogeneous outer layer is visibly associated with the

![Figure 1. SEM images and size distributions of fibers before and after UV irradiation: a) Untreated PCL nanofibers, b) PCL nanofibers after UV treatment for 2 min, c) 4 min, and d) 6 min.](image-url)
photoinduced decomposition of the PNIPAM polymer. This could be due to a stronger crosslinking of the polymer chains, which leads to contraction and shrinkage of the fibers and increased brittleness.\[26\] Fourier-transform infrared spectroscopy (FTIR) spectroscopy was used to provide additional information on the surface chemistry of the PCL/PNIPAM core–shell nanofibers. All FTIR spectra of fibers before and after UV irradiation show the characteristic bands of PCL (Figure 3). While the bands at 2929 and 2871 cm\(^{-1}\) can be assigned to the asymmetric and symmetric \(-\text{CH}_2\) stretching, the intensive band at 1720 cm\(^{-1}\) is related to the carbonyl group. Bands visible at 1242 and 1162 cm\(^{-1}\) can be related to the symmetric and asymmetric \(\text{C}–\text{O}–\text{C}\) stretching.\[27\] After UV treatment of the PCL core fibers, two new bands appeared. The bands at 1652 and 1548 cm\(^{-1}\) show the \(\equiv\text{C}–\text{O}\) stretching and \(\text{N}–\text{H}\) deformation vibration bands of the amide group of PNIPAM, respectively.\[14\]

The thermosensitivity of the PCL/PNIPAM electrospun nanofibers after UV photopolymerization was evaluated by water contact angle (WCA) measurements. Therefore, the wetting behavior of PCL/PNIPAM nanofibers at different temperatures (25 and 40 °C) below and above the LCST (32 °C) of PNIPAM was tested. A strong temperature dependency was observed for the wettability of core–shell fibers. At 25 °C, the static contact angles measured after UV treatment of 2 min and 4 min were 39° and 31°, respectively, indicating a high wetting and thus hydrophilic (CA < 90°) behavior (Figure 4a,b). However, after the temperature was raised to 40 °C, the WCAs of PCL/PNIPAM composite fibers after 2 min and 4 min of UV exposure increased to 119° and 131°, respectively, which proved a change to hydrophobic surface behavior (CA > 90°) of the fibers (Figure 4c,d). The switchable wettability of PCL/PNIPAM core–shell fibers thus indicated a collapse of PNIPAM chains upon temperature increase above the LCST, which can be useful, for instance, for triggering a temperature-induced release of loaded therapeutics.

To test the biocompatibility and possible applicability of PCL/PNIPAM core–shell nanofibers as drug delivery systems, cell viability tests of HEK 293 cells in the presence of electrospun nanofibers were conducted by incubating the fiber mats with cells for 24, 48, and 72 h before MTT assays were accomplished. Even after long incubation times of several days, no significant decrease in cell viability was observed (Figure 5); instead, values remained between 98% and 91% that confirmed the low cytotoxicity of the nanofibers and ruled out the presence of any residual
solvents or contaminants that could be detrimental for cell viability. These results and observed high biocompatibility of PCL and PCL/PNIPAM fiber mats are also in accordance with previous studies.\cite{28,29}

2.2. Drug Loading and Release

To demonstrate the suitability of as-prepared core–shell fibers for drug delivery applications, the antibiotic drug doxycycline hyclate (Doxy) was loaded as a model drug and release studies were conducted between 25 and 40 °C to evaluate the influence of the thermosensitive PNIPAM shell. The drug release profiles at 25, 37, and 40 °C in PBS demonstrated a clear temperature dependency (Figure 6) with the general trend being similar for all samples. Initially, a characteristic burst release took place, followed by a slow sustained release of the drug. During release experiments were conducted at 25 °C; thus, below the LCST of PNIPAM, the polymer chains were present in stretched form, allowing for complete wetting of the hydrophilic shell with the surrounding PBS solution. This can be observed and explained by a rapid outward diffusion of Doxy from the polymer matrix for the first 8 h (almost 62%), followed by a slow release over a period of 13 h (about 20%) (Figure 6a). At 40 °C and hence above the LCST of PNIPAM, the polymer switches to a hydrophobic behavior, so the hydrogen bonds between PNIPAM chain and water molecules were destroyed, which led to a deformation of the shell structure of the PCL/PNIPAM nanofibers.\cite{30} As Doxy molecules have to overcome the PNIPAM barrier when diffusing from the inside (core) to outside (medium), significant differences in release kinetics of drug molecules were observed for all measured samples. In fact, release profiles correlated well with the UV exposure time, as this altered the diameter of the PNIPAM shell as well as the coarsening of the polymer, thereby hampering the Doxy diffusion (Figure 6c). Therefore, highest release rates were observed for drug-loaded nanofibers before UV treatment (68%). After short UV treatment (2 min) of PCL/PNIPAM nanofibers, a cumulative Doxy release of 57% was observed upon 8 h incubation, whereas this value was reduced to 46% when 4 min UV irradiation time was used. Under physiological conditions at 37 °C, release rates were similar to 40 °C, and the differences due to varying PNIPAM shell thicknesses were less pronounced (Figure 6b), which indicated the potential for a high but sustained release at body temperature.

2.3. Antibacterial Activity of PCL/PNIPAM Core–Shell Electrospun Nanofibers

The antibacterial activity of produced nanofibers was tested against Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*) bacteria. Agar diffusion tests (Kirby Bauer test) are commonly used to evaluate the effectiveness of an antibacterial material against bacteria in a growth culture.\cite{32} All samples were tested at 37 °C for 24 h incubation.

Figure 6. Left: Doxy release profiles from electrospun nanofibers before (black squares) and after (2 min [red circle] and 4 min [blue triangle]) UV treatment at a) 25 °C, b) 37 °C, c) and 40 °C, respectively. Right: Correlation of cumulative Doxy release profiles at different incubation temperatures with varying UV crosslinking times. All release profiles initially follow a pseudozero-order kinetic (pink), which turns into a first-order kinetic (green).
Pure PCL fibers mats were used as control for this antibacterial test. As shown in Figure 7, no inhibition of the bacterial growth by PCL fibers was observed. In case of core–shell PCL/PNIPAM nanofibers, release of Doxy molecules resulted in growth inhibition of both Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria.

The observed inhibition zone of the PCL/PNIPAM fibers exposed for 2 min to UV light was larger for both bacterial strains compared with the PCL-PNIPAM fibers treated for 4 min with UV light. These results correlated well with the results of the in vitro release studies of the antibiotic, in which less Doxy was released by PCL/PNIPAM after 4 min UV irradiation compared with nanofibers polymerized under 2 min of UV treatment. The significant increase in the inhibition zone for S. aureus is presumably related to the higher sensitivity of these bacteria compared with the more resistant E. coli strain to Doxy.\[13\]

3. Conclusion

Core–shell nanofibers based on immiscible blends of biocompatible polymers were successfully produced by coaxial electrospinning to demonstrate high versatility in the tuning of the crosslinking time. The stimuli–responsive nature of PCL/PNIPAM core–shell nanofibers reported here was exploited to encapsulate a water-soluble drug (Doxy). The efficacy of dual functionality based on temperature-dependent solubility of nanofiber mats and antibiotic effect of the drug was verified by drug delivery and antibacterial activity studies, respectively. The sustained release observed in the coaxial fibers is controllable through the degree of crosslinking of the photopolymerizable shell, which was regulated by the duration of photopolymerization. Results obtained in this study demonstrate that the conformation and thickness of the PNIPAM shell can be controlled through UV irradiation time to control the drug release rates. Indeed, the released antibiotic molecules were potent enough to significantly inhibit the growth of both Gram-negative E. coli and Gram-positive S. aureus bacteria. These results demonstrate the enormous potential of nanoeengineered biomaterials in unifying the prerequisite of biocompatibility with smart properties such as stimuli–responsive delivery of drugs with a stable and sustained profile.

4. Experimental Section

Materials: Polycaprolactone (PCL, MW 70,000-90,000 g mol\(^{-1}\)), N-isopropylacrylamide (NIPAM), 1-hydroxyacyclohexyl phenyl ketone and N,N'-methylenebisacrylamide (MBA), doxycycline hyclate (Doxy), 2,2,2 trifluoroethanol (TFA), dimethyl sulfoxide (DMSO), and 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2-H-tetrazolium bromide (MTT) were purchased from Sigma Aldrich. All chemicals were used as received without further purification.

Fabrication of Drug-Loaded PCL Core Nanofibers: About 12.5% (w/v) PCL solution was prepared by dissolving PCL granules in TFE under ambient conditions for 3 h. After that, the antibiotic drug doxycycline hyclate (Doxy) and the PI 1-hydroxyacyclohexylphenylketone in ratio (0.4: 0.2: 1, w(Doxy): w(Pi):w(PCL)) were added to the polymer solution and stirred for over 5 h for complete dissolution. The flask was covered with aluminum foil to protect the solution from light. The electrospinning apparatus (IME Technologies, Netherlands) was equipped with a high-voltage generator and a precision syringe pump. The solution was loaded into a 5 mL syringe with a metal needle of 0.5 mm inner diameter. The flow rate of the solution was maintained at 10 \(\mu\)L/min, the applied voltage was 14 kV, and the distance between the needle and the collector was set to 10 cm. The nanofibers were collected on a cylindrical target covered with aluminum foil and dried at 25 °C under reduced pressure for 24 h to remove any residual solvent.

Fabrication of Core–Shell PCL/PNIPAM Nanofibers via UV Photopolymerization: The monomer NIPAM (0.2 g) and crosslinker MBA (4 g) (with the molar ratio NIPAM: MBA 1:100) were dissolved in 100 mL deionized water and stirred for 2 h to obtain a homogeneous solution. After that, the previously prepared electrosun PCL mat was fully immersed in the solution before its irradiation with a UV lamp (Helios Italglass, 170 Watt) for different time periods (2 min, 4 min, 6 min) to initiate the polymerization process for obtaining PCL/PNIPAM core–shell fibers. The produced mat was washed three times with deionized water to remove residual monomers and dried under reduced pressure for 24 h.

Characterization of Nanofibers: The nanofibrous morphologies were investigated using a SEV Nano Nova SEM 430 with an accelerating voltage of 30 kV after coating the polymer fiber mats with gold. Average fiber diameters and standard deviations were analyzed using an image analysis program (Image J). The core–shell structure of the fibers after UV photopolymerization was investigated using TEM (Zeiss LEO 912) operated at an acceleration voltage of 120 kV. The surface chemistry of the scaffold was characterized by attenuated total reflection FTIR spectroscopy (Perkin Elmer spectrum 400), recording transmittance of each sample between 4000 and 400 cm\(^{-1}\). Wettability of crosslinked PCL/PNIPAM scaffolds was evaluated by measuring the WCA using a video camera instrument (DSA 100, KRUESS GmbH, Germany). All WCA experiments were conducted three times for each analyzed sample under similar experimental conditions.

Cell Culture Experiments and MTT Assay: Human embryonic kidney HEK 293 cells (Life Technologies GmbH, Darmstadt, Germany) were cultured at 37 °C in a humidified atmosphere of 5% CO\(_2\) in air, in 125 cm\(^2\) tissue culture flasks containing 15 mL Dulbecco’s-modified eagle medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA). Cytotoxicity of crosslinked nanofiber mats was determined by MTT assay. For this purpose, the fibrous samples were cut into 1 x 1 cm\(^2\) pieces, which were placed in 12-well plates (Cellstar, Greiner Bio-One GmbH, Germany), whereas coverslips were used as control. Following this, 300 \(\mu\)L of HEK 293 cells with a density of 75 x 10\(^4\) cells mL\(^{-1}\) were seeded on the sample mats and cultured for 24 and 48 h, respectively. The MTT assay was used to determine the proliferation rate of cells. About 30 \(\mu\)L of MTT solution (5 mgmL\(^{-1}\) in phosphate buffered saline (PBS), pH = 7.4, Biowest) was added to each well and incubated at 37 °C for 3 h, before the solution was carefully removed. Formed purple formazan crystals were dissolved by adding 150 \(\mu\)L DMSO to each well. Finally, absorbance of the solution was measured (ELX800, BioTek Instruments, USA) at 490 nm, using 630 nm as reference wavelength. Each sample was measured in triplicates.
Obtained mean values were analyzed relative to those obtained by the control group.

Release Profiles of Doxy from Electrospun Nanofiber Mats: Release of Doxy from the electrospun fibers was monitored by UV–vis spectroscopy (Perkin Elmer UV/VIS Lambda 950) by comparative analysis of the peak observed at a wavelength of 275 350 nm. For this purpose, 1 × 1 cm² pieces of the nanofiber mats were transferred into 5 mL PBS and placed in a shaking water bath (SW22, Julabo) at different temperatures (25, 37, and 40 °C). At specific time intervals (1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, and 28 h), 3 mL of the supernatant was withdrawn and directly replaced with 3 mL of fresh PBS to maintain the same conditions. UV–vis measurements of the supernatant were carried out and then converted to the Doxy concentration according to the standard calibration curve of pure Doxy in PBS medium. The calibration curve of Doxy was established by measuring the absorbance at 275 nm of a serial dilution series with known concentration of Doxy in PBS. The cumulative amount of released Doxy was calculated as a function of time.

Antibacterial Evaluation of Doxy-Loaded Electrospun Nanofibers: The antibacterial activity of Doxy-loaded PCL/PNIPAM fibers was determined using a modified disc diffusion agar assay. Standard strains of bacterial culture of Staphylococcus aureus (S. aureus, ATCC 25 923) and Escherichia coli (E. coli, ATCC 25 922) were obtained from Institute for Microbiology, Immunology and Hygiene, University Hospital Cologne, and used as model bacteria for this study. The bacterial inoculum, made in saline solution (0.45% NaCl), was first adjusted to 0.5 McFarland standard (Vitek Desichek, BioMerieux) and then uniformly spread using a sterile cotton swab on a sterile Petri dish Mueller–Hinton (M–H) agar. The circular pieces of the nanofiber mats was monitored by UV/vis spectroscopy and controlled release, nano

Keywords
antibiotics, controlled release, nanofibers, thermoresponsive polymers

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Conflict of Interest
The authors declare no conflict of interest.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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