# A Novel Controlled Release Drug Delivery System for Multiple Drugs Based on Electrospun Nanofibers Containing Nanoparticles

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**ABSTRACT:** This study describes development of a novel controlled drug release system for multiple drugs, it consisted of Chitosan nanoparticles/PCL composite electrospun nanofibers with core–sheath structures. Two model agents' rhodamine B and naproxen were successfully loaded in the core and sheath region respectively. The behavior of these two agents demonstrated a good controlled release and temporality, providing a new way to obtain program or temporality release for multiple agents. Particularly, this is potential applications in the field of tissue engineering, sutures and wound dressings. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:4805–4811, 2010

**Keywords:** multiple drugs; controlled release; electrospun nanofibers; nanoparticles; core–sheath structures

## INTRODUCTION

Electrospinning has proven to be a simple, versatile, and useful technique for fabricating nanofibers from a rich variety of functional materials. In the past few years, we have witnessed tremendous research progress in understanding electrospinning mechanisms,[1–6] morphology or secondary structures,[7–13] as well as directed orientation.[14–16]

Electrospun fibers possess high surface area to volume or mass ratio, small inter-fibrous pore size with high porosity, as well as vast possibilities for surface functionalization. Furthermore, the simplicity of electrospinning process itself can also provide the ability being convenient to incorporate therapeutic compounds into the electrospun fibers for preparing useful drug delivery system. Since Kenawy took the lead in exploring electrospun fiber mats as drug delivery vehicles using tetracycline hydrochloride as a model drug in 2002,[17] scientist community has started to pay close attention to this field. Up till now, many related studies have already described preparation of drug-loaded electrospun fibers using a number of molecules including antibiotics, chemotherapeutics, as well as vitamins.[18]

Ideally, drug delivery systems should allow multiple drugs to be safely loaded within the polymeric or other matrix and enable the release of each drug to be independently controlled. This is a common strategy for overcoming multi drug resistance (MDR) in cancer therapy or other complex diseases. Recently, Jabr-Milane et al.[19] reviewed the recent progress of combination therapy use of all kinds of nano-carries as multi-agent delivery system. While, as for polymeric nanofibers' carriers, at present, most studies have focused on embedding a single or multiple drugs into the same polymeric nanofiber matrix. Results of these studies showed that a controlled independent release of each drug in nanofibers cannot be achieved. This is mainly because the release profile depends on the diffusion pathway and degradation rate of embedding materials. Mostly, the above two factors are not distinct for the multiple drug embedded into the same matrix.

Recent studies have demonstrated that polymeric,[20,21] gold,[22] and silica[23] nanoparticles can be successfully packed together and formed into core–sheath nanofibers with chainlike structures. It is noteworthy that electrospun fibers with core–shell structures can provide the possibility to encapsulate two different drugs or biologically active agents within the core–shell structure at the same time. Jo et al.[20] demonstrated an electrospinning method for producing core–sheath nanofibers that is composed of an array of different colloids in which

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different active agents are loaded. The results showed that the process enables independent control over the release of each drug by adjusting the physical and chemical properties of the colloids in the core, leading to a programmed release of multiple drugs. Similarly, Dong et al.[21] have also successfully fabricated biocompatible polymeric electrospun fibers with the encapsulation of two distinct biological components-polyvinyl alcohol (PVA)/dextran and PVA/bovine serum albumin (BSA) nanoparticles at the same time. Although this study did not deal with the release characteristics of drugs, this method could provide a possible way to obtain controlled release of multiple drugs without fail.

It is different from above two studies that this communications demonstrates a novel controlled release drug delivery system for multiple agents based electrospun nanofibers with core–sheath structures, the core is composed of chitosan encapsulated drug, and another drug directly embedded into sheath of fibers at the same time. Because these two drugs are loaded into distinct different polymer matrix, the difference of diffusion pathway could cause a controlled release of multiple drugs. But even more important, the method also provides the effective way to independent control the release behavior of each drug.

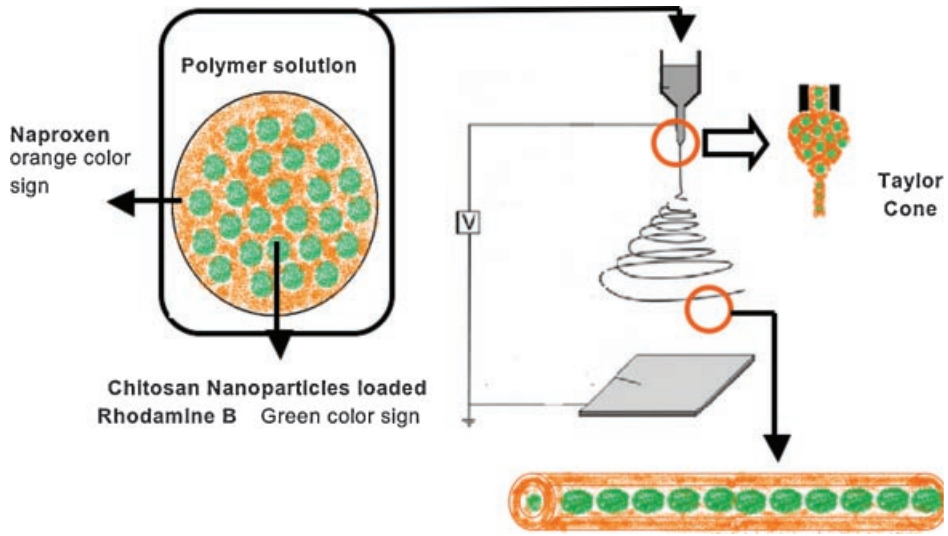
## EXPERIMENTAL PART

Poly (ε-caprolactone) (PCL, $M_w = 80000$) is purchased from Daigang (Ji'nan, China). Chitosan (Cs, $M = 100$–300 kDa, Degree of deacetylation = 85%) and sodium tripolyphosphate (TPP) are commercial products of Kelong Regent Factory (Chengdu, China). Rhodamine B was purchased from Songon (Shanghai, China). Naproxen was purchased from Yinhe (Wuhan, China).

The chitosan nanoparticles were obtained based on ionic gelation of TPP with chitosan.[24] Briefly, chitosan was dissolved at 2.0 mg/mL in 0.5% (v/v) acetic acid, and Rhodamine B was added in the solution, and then raised to a suitable pH (4.3) with 1 mol/L NaOH. The chitosan nanoparticles were obtained upon addition of 20 mL TPP solution (0.7 mg/mL) into 50 mL chitosan solution under mildly mechanical stirring at room temperature. Nanoparticles were collected by centrifuge at 13500 rpm at 4°C for 70 min and washed with water to remove any residual free Rhodamine B, then redispersed in DMSO solution.

Poly (ε-caprolactone) was dissolved in chloroform–methanol (3:1, v/v) to prepare an 11 wt% solution. Naproxen (1%) and nanoparticles (25%), both with respect to the weight of polymer, as well as 2% Tween-80 (v/v) was added into PCL solution under constant stirring for 2 h, this PCL suspensions was used to prepare the nanoparticle/nanofiber composite by electrospinning (Scheme 1). Scheme 1 presents a schematic illustration of the process of this study. A polymer solution with uniformly distributed Chitosan nanoparticles is electrospun through a single nozzle. As a result, the core/sheath fibers consisting of nanoparticles core can be produced. In addition, multiple agents were loaded into the Chitosan nanoparticles (Core) and sheath respectively to provide independent control over the release of each agent.

The spray rate of the solution from the syringe was controlled 1.5 mL/h by using a syringe pump. The




**Scheme 1.** Schematic illustration of method for preparing core–sheath nanofibers containing chitosan nanoparticles encapsulating Rhodamine B (as core). Naproxen was embedded into the sheath. Multiple agent loading in different site of this core–sheath nanofiber can provide independent control over the release of each agent.

voltage (Tianjin High Voltage Power Supply Company, Tianjin, China) applied to the needle of the syringe was 16 kV and the distance between the needle tip and a grounded aluminum foil as collectors was kept at 15 cm. A scanning electron microscope (SEM, TESCAN, VEGAII, LMU, Brno, Czech) was used to observe the surface morphology of the electrospun fibers. Its accelerating voltage was 10 kV.

In order to detect the distribution of nanoparticles in nanofibers, the nanoparticles were labeled by Fluorescein isothiocyanate (FITC). The preparation of FITC-labeled nanoparticles was according to a previously reported procedure.[25] Briefly, the precipitated nanoparticles were redispersed into 2 mL DMSO solution followed by the addition of 0.5 mol/L NaOH to adjust the pH > 6.5. FITC was dissolved in DMSO at 2.5 mg/mL, and was slowly added to the suspension of chitosan nanoparticles. The reaction proceeded in the dark at room temperature under constant stirring for 3 h. The labeled nanoparticles were obtained by centrifugalization. The FITC-labeled nanoparticles/nanofibers composite was obtained *via* electrospinning as mentioned above. Fluorescence optical microscope (Leica, DMI4000B, Wiesbaden, Germany) and a laser scanning confocal microscope (LSCM) were used to evaluate the distribution of FITC-labeled nanoparticles in the electrospun composite nanofibers.

During the release studies, the composite nanofiber mat was first cut into pieces with the mass of 100 mg, and then placed in a vial filled with 20 mL of prewarmed phosphate buffer saline (PBS). The vial was incubated at 37°C protected from light in a thermostated shaker. At appropriate intervals, 2 mL of release medium was removed for sampling and replaced with an equal volume of fresh medium. The concentrations of Rhodamine B and Naproxen were determined by dial-wavelength spectrophotometry respectively.

## RESULTS AND DISCUSSION

For successfully electrospun core–sheath nanofibers containing nanoparticles, it is necessary to produce the chitosan nanoparticles encapsulating Rhodamine B before electrospinning. Figure 1a and b shows a typical SEM image and size distribution of these nanoparticles prepared using method mentioned in Experimental Part Section, Figure 1b shows that nanoparticles exhibited a uniform diameter of 476.5 nm, and the average size of 388 nm.

The process for the fabrication of Chitosan nanoparticles/PCL composite electrospun nanofibers with core–sheath structures is depicted in Scheme 1. Because the chitosan nanoparticles are immiscible

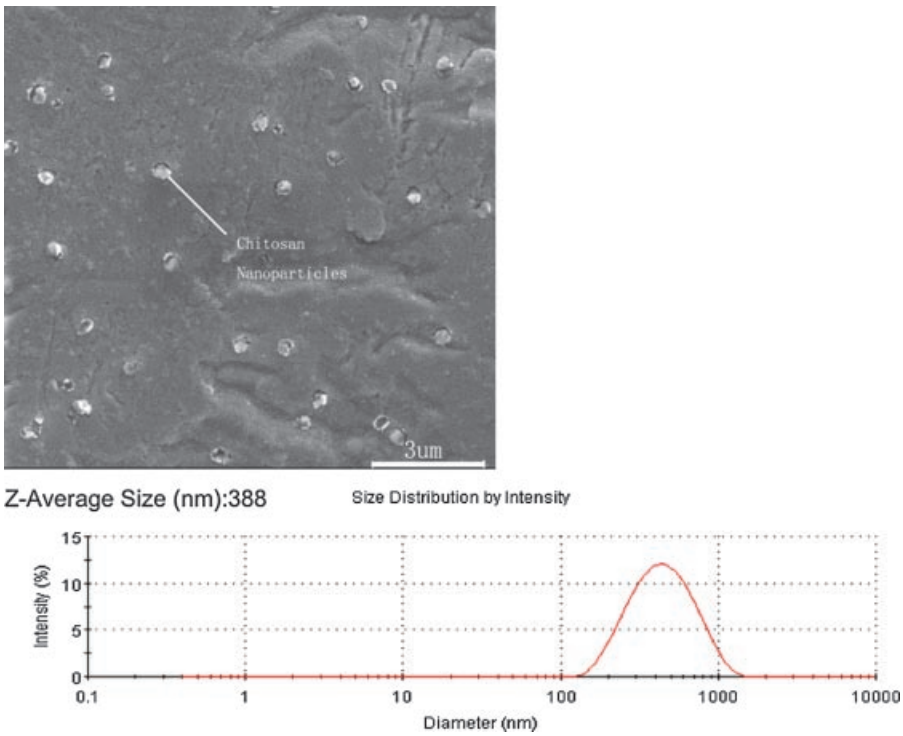



**Figure 1.** a: Typical SEM image of Chitosan nanoparticles containing Rhodamine B. b: Size distribution of Chitosan nanoparticles containing Rhodamine B; Scale bar is 3 μm.

with PCL solution, their composite solution formed stable and uniform emulsion after addition of Tween-80. Core–sheath nanofibers were successfully obtained, and the nanoparticles were linearly packed and formed chainlike structures along the fiber direction (Fig. 2b). SEM images (Fig. 2c and d) further accorded with this observation. However, we found that those nanofibers showed a wide distribution of diameter (from 100 to 500 nm) and lower particle–particle attractive interaction (Fig. 2d white arrow sign). We think that, the aggregation of nanoparticles in the process of disperses into PCL solution and electrospinning lead to this phenomenon.[26,27] Checking nanoparticles/PCL composite solution drop-cast onto glass slide using of LM (Fig. 2a), the result demonstrated the appearance of aggregation occurs in the process disperse, and formed microspheres. Therefore, controlled the adapted concentration of nanoparticles, the nanoparticles can be phase separated to be packed together at the inner region of fibers.

In order to directly visualize the nanoparticles located in PCL fibers, we introduced a fluorescently (FITC) labeled chitosan nanoparticle, and then imaged using Fluorescent and Confocal laser scanning microscopy (CLSM). Figure 3a is a florescent image of nanoparticles-FITC/PCL nanofibers. It is clearly observed that the nanoparticles were linearly encapsulated by the PCL fibers and formed chainlike structures along the fiber direction. The CLSM image (Fig. 3b) further demonstrated the nanoparticles were encapsulated in the central region of the fibers. As white arrow shown in the Figure 2a, incandescent florescent spot, the aggregation of nanoparticles cause these chainlike structures to have little blemishes.

Figure 4a shows the release of rhodamine B and Naproxen from core–sheath nanofibers. The data showed that both demonstrated a controlled release as compared with free drug, and their accumulated drug release was 60.5% (Naproxen) and 18.1%(rhodamine B) after 72 h respectively.

Apart from the physical and chemical properties of drug, its release after being embedded inside the polymer matrix is affected by rate of diffusion alone or by combined action of rate of diffusion plus embedded

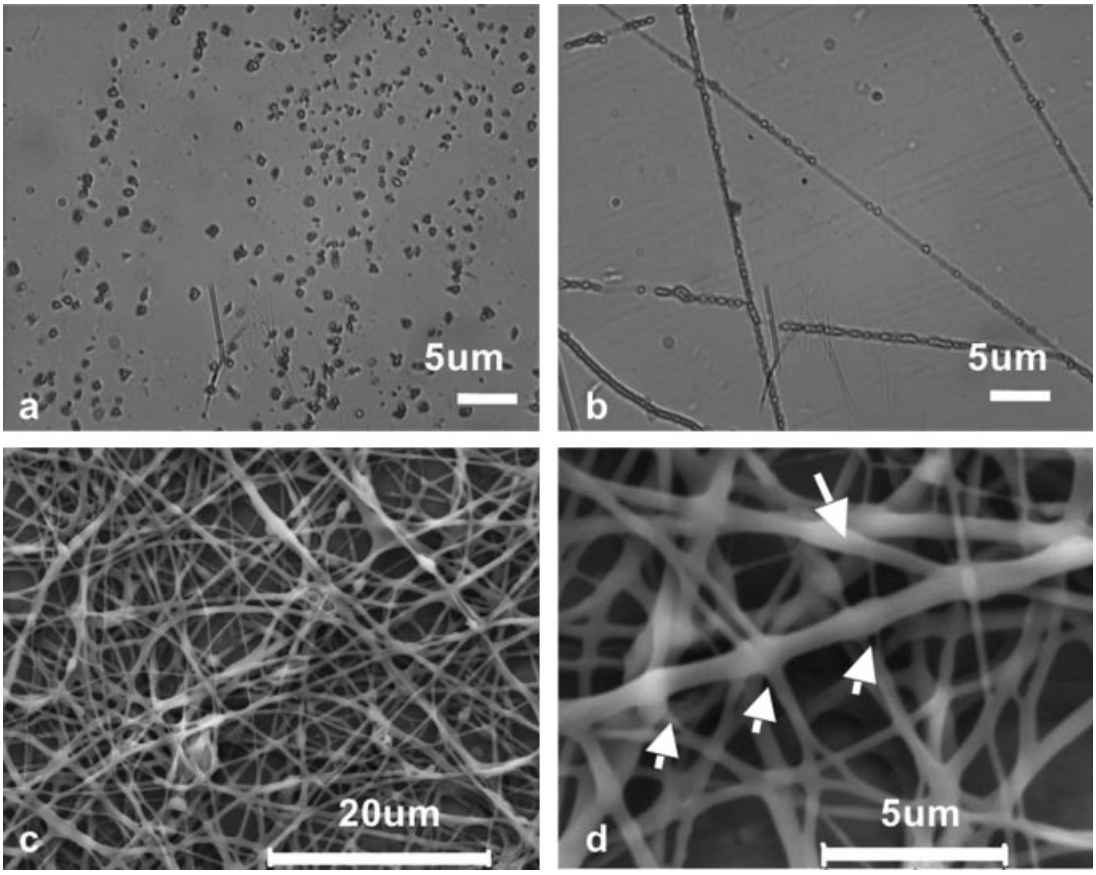



**Figure 2.** a: LM image (40×) of Chitosan nanoparticles encapsulating Rhodamine B. b: The LM image (40×) of Chitosan nanoparticles/PCL composted core–sheath electrospun fibers, Chitosan nanoparticles encapsulating Rhodamine B (Core) and PCL embedding Naproxen (Sheath). c and d: The SEM image of Chitosan nanoparticles/PCL composted core–sheath electrospun fibers.

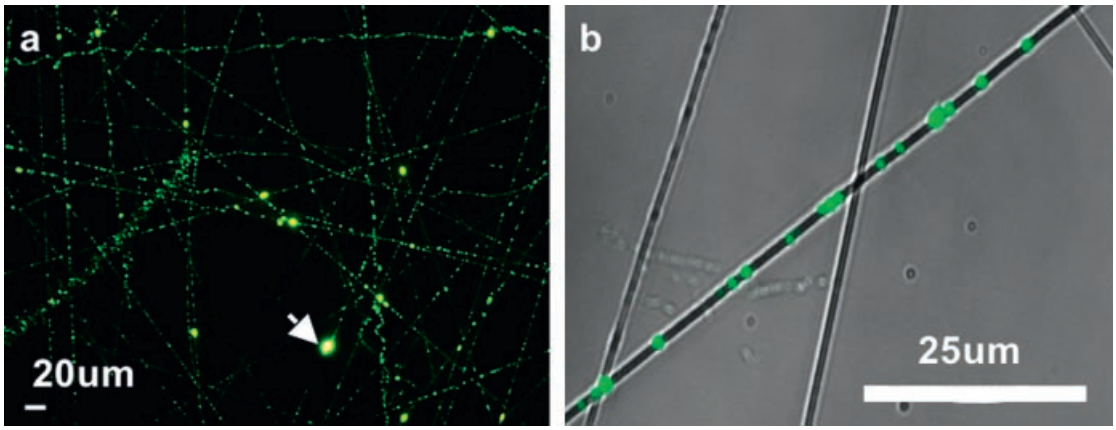



**Figure 3.** a: Fluorescent image of Chitosan nanoparticles-FITC/PCL core–sheath nanofibers. b: LSCM image of Chitosan nanoparticles-FITC/PCL core–sheath nanofibers; Chitosan nanoparticles labeled FITC shows green light.

material's degradation. In our study, two model agents: rhodamine B and naproxen were embedded respectively in the core and sheath region of nanoparticles/PCL composite core–shell fibers, naproxen embedded directly into the core–sheath nanofibers is released preferentially into the surrounding media *via* diffusion from nanofibers. However, rhodamine B loaded into nanoparticle endured double barriers of nanoparticle and nanofiber, which provides distinct diffusion pathway to these two drugs release from their location. Thus it can be deduced that the release profile of multiple agents in one same fiber could be temporality or programmed.

As expected the release profile exactly agree with our anticipate, as shown in the Figure 4a, the accumulated release of naproxen already reach 18% in sharp contrast to rhodamine B 4.3% in the first 1 h. Even after 4 h, the accumulated release of rhodamine B still is just 11.6%, meanwhile naproxen release rapidly increased up to 39.6%. Based on this data a scheme of diffusion pathway is proposed (Fig. 4b), depicting longer diffusion pathway of rhodamine B located in core compared with naproxen located in sheath. We think that the permeability distinction coefficient between chitosan with PCL also enhanced this difference of diffusion pathway. Wijaya et al.[28] obtained selective release of two distinct DNA oligonucleotides from two different DNA-Nanorod (NR) conjugates *via* selective laser-induced melting of NRs. Because laser fluence governs the degree of NR melting, yield, and specificity of DNA release, the controlled release can be externally tunable.

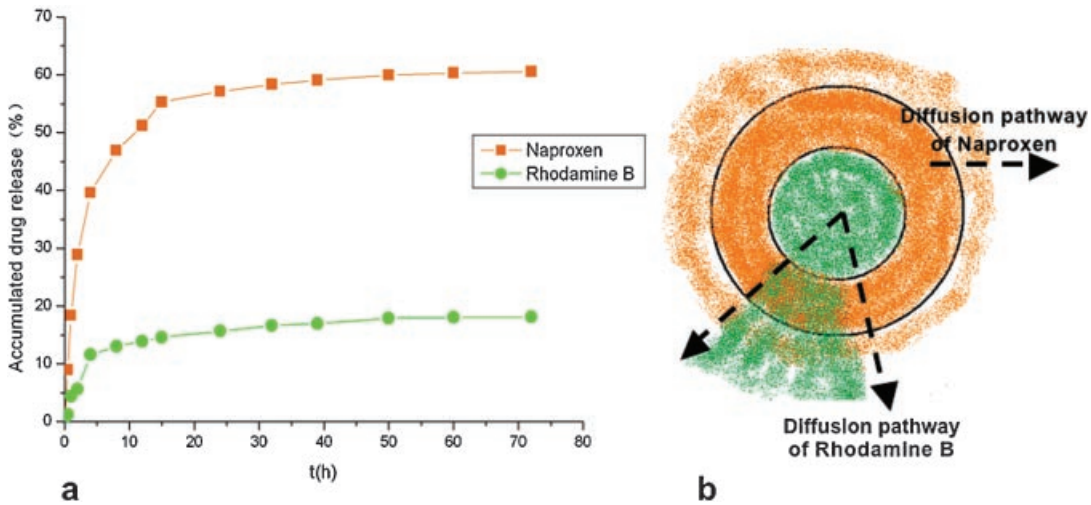



**Figure 4.** a: Release behavior of Rhodamine B and Naproxen from Chitosan nanoparticles/PCL electrospun nanofibers with core–sheath structures. b: Scheme of diffusion pathway of Rhodamine B and Naproxen from core–sheath electrospun nanofibers, Orange color—Naproxen, and Green color—Rhodamine B.

Differentiation from aforementioned NRs drug delivery, in our study, naproxen and rhodamine B were loaded in different regions of a same nanoparticles/PCL composite fiber respectively, our results indicate that it is possible to obtain anticipative temporality release through the control of material properties of core and sheath independently.

To assess the mechanism of drug release, the linearization of accumulated release against the square root of time was evaluated.[29] When accumulated release of naproxen was less than 0.6, the linear regress of Higuchi kinetics was $r = 0.8774$, as was $r = 0.8816$ for rhodamine B. This indicates that the release of naproxen and rhodamine B from nanoparticles/PCL composite core–shell fibers matrix over this time period agreed with Higuchi kinetics, and was diffusionally controlled. However, the abovementioned linearization of Higuchi kinetics was not significant linear in comparison to other research that single drug was loaded in the electrospun nanofibers.[29,30] This suggests that the release behavior of each drug from multi-drug loaded system could be affected each other, this interaction of multi drugs resulted in that it is difficult to use an existing release kinetic to fit their release profile.

During release from 1 to 72 h, the distinct release behavior of these two drugs indicated that this method of preparing drug-loading electrospun nanofibers with core–sheath structures can successfully achieve controlled release of multiple agents loaded into the same system. It is worth noting that the release of each drug from this core–sheath nanofiber loaded multiple drugs might be further controlled by adjusting the diffusion pathway or degradation of polymer. Concretely speaking, diffusion pathway can be change *via* adapted core or sheath materials, size of nanoparticles and nanofibers, as well as drug-loading rate. In addition, the degradation rate of core and sheath materials independently also provide a possible way to program or temporality release of multiple drugs.

## CONCLUSION

In summary, we have demonstrated a one-step fabrication of core/sheath fibers using of a single-nozzle setup. The uniqueness of this study results that the polymeric nanoparticle loaded drugs can be encapsulated in the core region of electrospun fibers and formed chainlike structures, moreover, loading different drugs in core region and sheath region at the same time respectively, can provide distinct release behavior, which enables a program or temporality release for multiple agents. This is of particular interest in the field of tissue engineering, where spatial differentiation, program, as well as temporality of active agents are needed to control for cell proliferation, differentiation, and functionalized.

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