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# Controlled release of liposome-encapsulated Naproxen from core-sheath electrospun nanofibers

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ABSTRACT

Naproxen (NAP) loaded nanofibers of different structures have been successfully prepared by electrospinning. The structures of the nanofibers are NAP and cellulose acetate (CA) mixed nanofibers (NF-1), nanofibers with NAP/CA mixed core and CA sheath (NF-2), and NAP loaded liposomes and sodium hyaluronate (HA-Na) mixed core with CA sheath (NF-3). The structure and morphology of the nanofibers were characterized and the drug release behaviors were investigated. It was found that NAP can disperse in the HA-Na or CA matrix in molecular level without formation of NAP crystals and dimers. The drug release behaviors of NF-1 and NF-2 show a non-Fickian diffusion mechanism, while the NF-3 shows a specific drug release behavior with a burst release within 8 h followed by a sustained drug release for 12 days. The particular two-stage drug release behavior of NF-3 nanofibers offers the materials promising applications as wound dressing materials.



## 1. Introduction

Nanofibers have been extensively studied as tissue engineering scaffolds for decades. Owing to high specific surface area ratio and high porosity, drug-loaded electrospun nanofibers have found clinical applications in wound dressings, orthopedics and tissue regeneration (Holzapfel et al., 2013; Liu et al., 2012; Schiffman & Schauer, 2008). The development of electrospinning techniques has brought forward multiple approaches to fabricate various nanofibers from synthetic and natural polymers (Gupta, Elkins, Long, & Wilkes, 2005; Ohkawa, Minato, Kumagai, Hayashi, & Yamamoto, 2006; Zhang & Chang, 2008). Bio-friendly nanofibers loading various drugs and bioactive molecules thus are widely studied as drug delivery systems (Buschle-Diller et al., 2007; Xie et al., 2013). Among the challenges for nanofiber drug carriers, the most important one is to control the burst drug release. The burst drug release is helpful to suppress the infection and pain of a wound during the initial stage. However, sustained drug release is more important for efficiently protecting the wound for long periods without changing the wound dressing, which is good for wound healing and alleviates the pain caused by dressing changes.

Nanofibers produced by directly electrospinning from drug/polymer mixed solution have drugs dispersed in the polymer matrix, which usually display burst drug release behaviors via Fickian or non-Fickian diffusion (Tungprapa, Jangchud, & Supaphol, 2007; Yu, Yu, Chen, Williams, & Wang, 2012b). Various approaches have been tried to control the burst drug release from nanofiber mats, among which coaxial electrospinning has proved to be an effective one. In the coaxial electrospinning approach, core-sheath nanofibers with drugs embedded in the core matrix can be produced. It was reported that ketoprofen-loaded cellulose acetate (CA) fibers coated with blank CA sheath have no burst release and can provide a zero-order in vitro drug release profile over 96 h (Yu, Yang, Li, Lu, & Zhu, 2012a). Sustained drug release from electrospun nanofibers can also be achieved by encapsulating drug-loaded vehicles in the electrospun nanofibers (Qi et al., 2010; Shao et al., 2011; Song, Wu, & Chang, 2012). It was found that burst release of protein can be avoided by embedding protein-loaded

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liposomes in the core matrix of core-sheath nanofibers (Mickova et al., 2012). The incorporation of drug-loaded (nano)particles into electrospun fibers can also efficiently prolong the drug release time. (Coneski, Nash, & Schoenfisch, 2011; Hou et al., 2013; Koh, Carpenter, Slomberg, & Schoenfisch, 2013; Xie et al., 2013).

Desired drug-loaded wound dressings should have a burst drug release at the beginning and then a sustained drug release for a long period. However, no such dressings has been prepared. In this work, CA and HA-Na were used to prepare core-sheath nanofibers with the CA sheath and HA-Na core by coaxial electrospinning. Due to that HA-Na can stabilize liposomes by adsorbing on the surfaces (Quemeneur, Rinaudo, Maret, & Pepin-Donat, 2010), Naproxen (NAP) loaded liposomes were embedded in the HA-Na core of the nanofibers. The morphology and drug release behavior of the nanofibers were investigated and were compared with nanofibers of different structures. It was found that the approach in present work could successfully prepare nanofibers that have a burst drug release at the initial stage and subsequently a sustained drug release for a long period.

## 2. Experimental

### 2.1. Materials

Cellulose acetate (CA, $M_r$ = 29 kDa, DS = 2.4–2.5) was purchased from Sigma Aldrich (USA). Lecithin (PC, from Soybean) and sodium hyaluronate (HA-Na, $M_w > 10^3$ kDa) were supplied by Aladdin (China). Cholesterol (Chol) and naproxen (NAP) were purchased from Alfa Aesar (USA). Highly purified water was prepared using Milli-Q Water Purification System (Millipore, USA). Dimethyl sulfoxide (DMSO) was supplied by Beijing chemical Works and distilled before use. Other chemicals including chloroform, methanol, acetone and *N*,*N*-dimethylacetamide (DMAc) are all analytical grade and were used as received.

### 2.2. Preparation of NAP loaded liposomes

NAP loaded liposomes were prepared from PC, Chol and NAP using the conventional thin film hydration method. Briefly, 160 mg PC, 20 mg Chol and 90 mg NAP were added to a 250 mL round bottom flask and then were dissolved in 5 mL mixed solvent of chloroform and methanol (2:1, v/v). Thin lipid film was produced by evaporating the solvents using a rotary evaporator operated at 30 °C and 60 rpm. The dried lipid film was then hydrated with 5 mL water using the above conditions in a nitrogen atmosphere. After being swollen overnight, the dispersion was sonicated in an ultrasonic water bath for 1 h at room temperature to generate narrowly distributed NAP loaded liposomes. The resultant NAP loaded liposomes are in the size of intermediate-size unilamellar vesicles (IUVs) and are denoted as NAP-IUVs henceforce.

The encapsulation efficiency of NAP in the liposomes was defined by $Q_w$ (%) = $W_e/W_N \times 100$, where $W_e$ and $W_N$ are the mass of NAP encapsulated in the liposomes and the amount of NAP used during the preparation of NAP-IUVs. To determine $Q_w$ (%), the as-prepared NAP-IUVs dispersion was centrifuged 10 min at $10^4$ rpm to remove the un-encapsulated NAP, which is normally precipitated in aqueous solution. The supernatant was lyophilized and weighed to obtain the mass ($W_s$) of the NAP loaded liposomes. The mass of the encapsulated NAP was determined as follows. The lyophilized sample was first dissolved in 2 mL DMSO and then diluted to 100 mL by buffer solution (pH = 6.4). The concentration of NAP ($C_N$) in the resultant solution was determined by comparing the UV absorbance at wavelength of 262 nm to a calibration curve of UV absorbance intensity versus NAP concentration, by which the mass of the encapsulated NAP ($W_e$) in the samples was determined. The corresponding feeding mass of NAP ($W_N$) is half to that of pure liposomes, which can be calculated by $(W_s - 100C_N)/2$.

### 2.3. Preparation of NAP loaded nanofibers

Core-sheath nanofibers with CA sheath and HA-Na core were prepared as follows. NAP-IUVs-HA-Na aqueous solution and CA (17%, w/v) solution in acetone/DMAc (2:1, v/v) were used as the core and sheath fluids of coaxial electrospinning, respectively. The core fluid was prepared in advance by mixing NAP-IUVs dispersion with HA-Na aqueous solution (3%, w/v) in a bottle in the volume ratio of 1:2 using a vortex shaker. The needle was settled coaxially by two needles with inner diameters of 0.5 and 1.2 mm. Extruding rates were set at 0.05 and 0.37 mL/h for the core and sheath solutions, respectively. The voltage and the distance between the needle and the collector (aluminum foil) were set at 15 kV and 15 cm, respectively. The electrospinning was carried out at room temperature and a humidity of about 20%. The resultant nanofibers were denoted as NF-3.

For comparison, NAP loaded CA nanofibers were also prepared from NAP/CA (1.9%/19%, w/v) mixed solution in acetone/DMAc (2:1, v/v). The needle used has a inner diameter of 1.0 mm and the extruding rate was 0.5 mL/h. The electrospinning was operated at 12 kV with needle to collector distance of 15 cm. The resulted nanofibers were denoted as NF-1. Core-sheath nanofibers with CA sheath and NAP/CA core were prepared by coaxial electrospinning. The CA (19%, w/v) and NAP/CA (1.9%/19%, w/v) solutions were used as the sheath and core fluids, respectively. Coaxially settled needles with inner diameter of 0.7 and 1.4 mm were used and the fluid extruding rates were both 0.5 mL/h. The electrospinning was operated at 15 kV with needle to collector distance of 15 cm. The prepared core-sheath nanofibers were denoted as NF-2. The two coaxial needles used were different, because they were selected depending on the properties of the respective fluids to avoid plugging and to prepare desired nanofibers.

NAP content in the nanofibers was determined by UV absorbance. About 350 mg nanofibers ($W_1$) was dissolved in 2 mL DMSO. The solution was then diluted to 100 mL with buffer solution (pH = 6.4) that was prepared by dissolving potassium dihydrogen phosphate and sodium hydroxide in water. The concentration of NAP ($C_N$) in the resultant solution was determined by UV absorbance as described above, by which the mass of NAP in the nanofibers ($W_N$) was determined. The content of NAP in the nanofibers was calculated by $C$ (%) = $W_N/W_1 \times 100$.

### 2.4. Instruments and characterization

The morphology of the electrospun fibers was observed on a field emission scanning electron microscope (FESEM, JEOL 6700, Japan) operated at accelerating voltage of 5 kV and current of 10 μA. The samples on aluminum foil were sputter-coated with a layer of platinum using a sputter-coater (BAL-TEC, SCD 500, Switzerland) before observation. All the images were taken in secondary electron mode. Elemental analysis was performed on an energy dispersive X-ray spectrometer (EDS, Oxford, UK). Transmission electron microscope (TEM) observation of the nanofibers was carried out on a field emission transmission electron microscopy (FETEM, 2200FS, JEOL, Japan). The samples were prepared by fixing copper grids on the collector and collecting fibers during the electrospinning. The operation voltage of TEM was set 200 kV, and the images were recorded in bright field mode.

Differential scanning calorimetry (DSC) measurements were carried out on DSC Q2000 (TA Instruments, USA) in the range of 20–200 °C at the heating rate of 10 °C/min in a nitrogen atmosphere. Wide angle X-ray diffraction (WAXD) experiments were carried out on a D/max 2500 X-ray diffraction meter (Rigaku, Japan).