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Preparation of paclitaxel/chitosan co-assembled core-shell nanofibers for drug-eluting stent

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ABSTRACT

The paclitaxel/chitosan (PTX/CS) core-shell nanofibers (NFs) are easily prepared by co-assembly of PTX and CS and used in drug-eluting stent. The mixture solution of PTX (dissolved in ethanol) and CS (dissolved in 1% acetic acid water solution) under sonication will make the formation of NFs, in which small molecule PTX co-assembles with biomacromolecular CS through non-covalent interactions. The obtained NFs are tens to hundreds nanometers in diameter and millimeter level in length. Furthermore, the structure of PTX/CS NFs was characterized by confocal laser scanning microscopy (CLSM), zeta potential, X-ray photoelectron spectroscopy (XPS) and nanoscale infra-red (nanoIR), which provided evidences demonstrated that PTX/CS NFs are core-shell structures. The 'shell' of CS wrapped outside of the NFs, while PTX is located in the core. Thus it resulted in high drug loading content (>40 wt.%). The well-controlled drug release, low cytotoxicity and good haemocompatibility were also found in drug carrier system of PTX/CS NFs. In addition, the hydrophilic and flexible properties of NFs make them easily coating and filming on stent to prepare drug-eluting stent (DES). Therefore, this study provides a convenient method to prepare high PTX loaded NFs, which is a promising nano-drug carrier used for DES and other biomedical applications. The possible molecular mechanism of PTX and CS co-assembly and core-shell nanofiber formation is also explored.

Statement of significance: We develop a convenient and efficient approach to fabricate core-shell nanofibers (NFs) through the co-assembly of paclitaxel (PTX) and chitosan (CS). Results indicate that the co-assembled PTX/CS NFs have high drug loading content (>40 wt.%), low cytotoxicity, well-sustained drug release and good haemocompatibility. The PTX/CS NFs coated easily on stents with substrate surface uneven and irregular. Findings from this work provide a novel drug/polymer system, which would have potential application for drug-eluting stent (DES).

1. Introduction

Molecular self-assembly is common in nature [1,2]. It is the spontaneous and reversible organization of molecular units to ordered structures by non-covalent interactions [3–5]. In recent years, self-assembly is considered as an inspired approach for design and micro/nano-fabrication of new materials with molecular mechanism in nanomaterials and biomedical engineering [4,6,7]. Among different kinds of nanostructures formed by self-assembly, nanofibers (NFs) have attracted intensive attention in nanotechnology and biomedical engineering owing to easy and controllable preparation, wide range of applications in medicine, including drug delivery [8,9], tissue engineering [10] and medical textile materials [11].

Furthermore, chitosan (CS) is one of the most popular materials in the area of polymers used for drug delivery systems, and is by far the most applied natural polymer [12,13]. It is a nontoxic, biodegradable and biocompatible polysaccharide of β (1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine with the presence of reactive amino and hydroxyl functional groups in structure [14,15]. The attractiveness of drug carrier application relies on CS structural and biological properties including cationic character and solubility in aqueous medium [16]. Since many drugs have problems of poor

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stability, water insolubility, low selectivity, high toxicity and other side effects [17], clinical use of drug delivery systems are required for delivering drugs efficiently and achieving good therapeutic effects. These can be overcome by the nanoscale drug delivery systems [18–22]. CS nanostructures are considered as potential drug delivery system, which attracted increasing attention in biomedical applications [23,24].

Drug-eluting stent (DES) is a peripheral or coronary stent (a scaffold) placed into narrowed, diseased peripheral or coronary arteries that slowly releases a drug to block cell proliferation, which is also conceived as a specific drug delivery system combining with stent. It can improve the overall effectiveness of angioplasty and stenotic procedures performed on the cardiovascular system and other vessels within the body by delivering potent therapeutic drugs [25–27]. Paclitaxel (PTX) is one of Food and Drug Administration (FDA)-approved antiproliferative drugs used for DES, and PTX-eluting stents are widely used in clinical interventional therapy [28]. However, the clinical application of PTX is limited because of the poor water solubility and serious side effects [29]. In order to increase PTX therapeutic effects and reduce its side effects, the problem of solubility, stability, drug loading efficiency, sustained and controlled release of PTX should be solved before designing and fabricating a novel DES [30]. Various nanocarrier systems including nanoparticles, liposomes, micelles, bioconjugates and dendrimers have been developed [31]. The therapeutic agent incorporated into polymeric solution and then coating on stent is the conventional approach to make DES [32,33]. Thus, the proper choice of polymer, the effective way of drug encapsulation, and well-controlled drug release are three key issues in DES design and fabrication [34–36]. To fulfil the three requirements, the ideal coating should be capable of accommodate enough amount of drugs and controlled drug release at sufficient therapeutic level for several days, weeks or longer [37]. The coating is also required to be thin enough and not increase the profile of the stent obviously [38]. In addition, the biodegradable and biocompatible coatings are required to reduce side effects. The nanostructures of CS and its derivatives made by self-assembly were proved to be good carriers for drug delivery [39]. Different CS-based nanostructures have been prepared including micro- nanoparticles, micelles and nanofibers [40]. However, the nanofibers obtained from CS derivative and other polymers are usually got from complex chemical synthesis process [41,42]. The complexity of CS derivatives would result the uncertainty of medical applications and they are difficult to be approved by FDA in clinical application [43]. Some researchers used freeze-drying and electrospinning to get NFs [44], J. Nie et al. obtained fiber membrane by freeze-drying for tissue engineering and drug carriers [45,46]. B. Ding et al. prepared nanofibers through electrospinning technique to make gas sensors [47,48]. But for drug delivery systems, the freeze-drying method is not suitable due to difficulty in controlling the formation conditions including drug loading and well-defined morphology of carriers. Meanwhile, electrospinning is a sophisticated method of producing drug-loaded NFs with well-controlled core-shell structure and limited drug loading content. Compared with two methods above, self-assembly would be a convenient and reliable method for developing new nano-drug delivery systems. Thus, it is an interesting thing if PTX can co-assemble with polymer, and CS based drug delivery system made by assembly would be promising. Up till now, the core-shell NFs prepared by the co-assembly of CS and PTX have not been reported.

In this study, we develop a facile approach to fabricate core-shell drug encapsulated NFs through co-assembly of PTX and CS in mixed solution. The core-shell nanostructures were well characterized. The drug release behaviour, cytotoxicity, haemocompatibility of PTX/CS core-shell NFs and PTX/CS NFs coating properties were evaluated in vitro. This study provide a convenient approach to achieve nanoscale core-shell drug delivery system for PTX drug efficient-encapsulation and well-controlled release, which could have potential application in DES.

2. Materials and methods

2.1. Materials

The bare stents made by Ti-Ni shape memory alloy was purchased from Jiangyin Fasten-PLT Materials Science Company Ltd. These stents were with strut dimensions of 0.148 mm × 0.048 mm (width × thickness). Paclitaxel (PTX) was purchased from Boshi Biological Technology Co., Ltd. Chitosan (CS) powder (average Mw, 144 kDa (Table S1); deacetylation degree 79%), fluorescein isothiocyanate isomer I (FITC), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide (MTT), Triton™ X-100 and deuterated solvents for NMR were purchased from Sigma-Aldrich and used as received. Dextran (Mw, 40 kDa), glutaraldehyde (2.5%) and other used organic solvents were obtained from the domestic suppliers. The other reagents and solvents were of analytical grade and used as received unless mentioned. Dialysis bags (Shanghai green Bird Science & Technology Development Co., Ltd, MWCO: 3500 Da). Ultrapure water was used in all experiments.

2.2. Preparation of PTX/CS NFs and PTX/CS NFs coating stents

2.2.1. Preparation of PTX/CS NFs

The PTX/CS NFs (2:2) was fabricated by a one-step approach. First, PTX was dissolved in ethanol to get 2 mg/mL PTX solution, CS was dissolved in 1% acetic acid aqueous solution to get 2 mg/mL CS solution, then PTX and CS solutions were mixed of equal volume. Second, ultrasonication (KQ-500DE) was applied to the mixed solution for 20 min. The prepared PTX/CS mixed solution after ultrasonication was concentrated using Amicon Ultra-15 Centrifugal Filter Unit with 10 kDa molecular weight cutoff (Millipore). Then the concentrated solution was dialyzed in ultrapure water and the water was changed every 2 h for the first day and fourth the next two days. Finally, PTX/CS (2:2) NFs were harvested by freeze-drying. The free PTX which didn't join the co-assembly with CS would deposit at the bottom of the Centrifugal Filter Unit. The UV absorbance at 227 nm of the bottom solution was measured to determine the concentration of unassembled PTX, therefore the mass of PTX which co-assemble with CS can be obtained by calculation correspondingly.

Drug loading content (DLC) and drug loading efficiency (DLE) were calculated according to the following formulas:

$$\mathrm{DLC\,(wt.\,\%)} = \text{weight of loaded drug}/(\text{weight of loaded drug} + \text{weight of polymer}) \times 100\%$$

$$\mathrm{DLE\,(wt.\,\%)} = \text{weight of loaded drug}/\text{weight of drug in feed} \times 100\%$$

During the preparation of PTX/CS NFs, the concentration of CS is also important. So we keep the PTX concentration at 2 mg/mL and set the CS concentrations at 0.5, 1, 1.5, 2, 2.5 mg/mL respectively, in order to find out whether the CS concentrations can affect the formation of PTX/CS NFs or not. SEM was used to characterize the morphology of co-assembled NFs.

Then PTX/CS (1:2) NFs were be prepared by adjusting the mass concentrations of PTX (1 mg/mL) and CS (2 mg/mL) solutions. PTX (2.0) aggregates were also prepared by mixing 2 mg/mL PTX ethanol solution with 1% acetic acid aqueous solution of the same volume, then ultrasonication, ultrafiltration, dialysis and freeze-drying were performed with the same conditions as above.

In order to investigate the structure of PTX/CS NFs, fluorescent (fluorescein isothiocyanate, FITC) labelled CS (FITC-CS) was