International Journal of Pharmaceutics 512 (2016) 204–212

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Rationally designed particle preloading method to improve protein delivery performance of electrospun polyester nanofibers

Xue Sun[a], Ke Li[a], Sainan Chen[a], Bing Yao[a], Yifa Zhou[b], Sisi Cui[b,*], Junli Hu[a,*], Yichun Liu[a,*]

[a] Key Laboratory of UV-Emitting Materials and Technology (Northeast Normal University), Ministry of Education, Changchun, Jilin, 130024, China
[b] School of Life Sciences, Northeast Normal University, Changchun, Jilin, 130024, China

ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form 23 August 2016
Accepted 25 August 2016
Available online 25 August 2016

Keywords:
Protein
Polyester nanofibers
Particle preloading
Rational design

ABSTRACT

Particle preloading method by first loading proteins onto nano- or microparticles and then integrating these particles into electrospun polyester nanofibers has been widely used to encapsulate therapeutic proteins into polyester nanofibers. However, poor method design has resulted in unsatisfactory protein delivery performance. For example, the harsh conditions involved in preloading procedures damage the bioactivities of proteins, the improper integration leads to an uneven distribution of particles in nanofibers or insecure attachment of particles to nanofibers, producing uncontrolled protein release profiles. This study aimed to improve the protein delivery performance of polyester nanofibers by rationally designing a particle preloading method. Positively charged chitosan nanoparticles (CNPs) were used as carriers to adsorb negatively charged proteins in mild conditions and as primary barriers for protein release. The polar CNPs were then homogeneously dispersed in a polar polyester solution and subjected to electrospinning. Microscope observations indicated that CNPs were homogeneously embedded within polyester nanofibers. In vitro release behaviour and cell studies showed that proteins retained their bioactivity and could release from polyester nanofibers in a sustained manner for more than 4 weeks without any initial burst. Epidermal growth factor encapsulated in polyester nanofibers enhanced diabetic wound healing in vivo, demonstrating an application potential in biomedicine. Other properties of the nanofibers, including composition, wettability, cytotoxicity, and cell adhesion and spreading, were examined in detail as well.

1. Introduction

Aliphatic polyesters such as polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) represent an essential class of biomedical materials because of their biocompatibility, biodegradability, and tuneable physiochemical properties (Seyednejad et al., 2011; Cameron and Shaver, 2011). In particular, their electrospun nanofibers are widely used in the delivery of therapeutic protein for wound healing or tissue regeneration (Hu et al., 2014; Xie et al., 2008). Owing to the different solubilities of protein and polyester, strategies such as emulsion electrospinning (Li et al., 2006; Kim et al., 2007), coaxial electrospinning (Zhang et al., 2006; Liao et al., 2006), and chemical conjugation (Choi et al., 2008; Cho et al., 2010) have been used to encapsulate proteins in electrospun polyester nanofibers and control the release of proteins. However, the success of these strategies has been limited because of problems such as damaged bioactivity of proteins or complicated experimental setups.

A new approach, the particle preloading method, has been developed, and it presents several advantages over other protein-loading strategies (Ionescu et al., 2010; Xie et al., 2013; Wei et al., 2007; Gungor-Ozkerim et al., 2014; Lai et al., 2014). The particle preloading method includes two steps: (1) load proteins onto nano- or microparticles; (2) integrate particles into polyester nanofibers. The particle matrix protects proteins from the harsh conditions involved in electrospinning (e.g., high voltage, organic solvents) and functions as a preliminary release barrier controlling the protein release rate. Various proteins have been encapsulated into polyester nanofibers via preloaded particles and applications in wound healing and tissue engineering have been explored (Ionescu et al., 2010; Xie et al., 2013; Wei et al., 2007; Gungor-Ozkerim et al., 2014; Lai et al., 2014).

* Corresponding authors.
E-mail addresses: cuiss100@nenu.edu.cn (S. Cui), hujl100@nenu.edu.cn (J. Hu), ycliu@nenu.edu.cn (Y. Liu).

http://dx.doi.org/10.1016/j.ijpharm.2016.08.053
0378-5173/

However, to achieve the ideal protein delivery performance, rational design is still lacking for current preloading methods. Some methods involved harsh conditions such as an oil-water interface and organic solvents in the preloading step, causing proteins to lose their bioactivities (Xie et al., 2013). Some methods integrated particles with polyester nanofibers simply by mixing the two components. The particles could easily shed off of nanofibers because there were no effective interactions between them, resulting in the loss of proteins (Gungor-Ozkerim et al., 2014). In others, to integrate the preloaded particles with polyester nanofibers, the authors dispersed particles in organic solutions of polyester and the suspensions were then subjected to electrospinning. Because the dispersion of particles in polyester solution was uneven, the distribution of particles in the polyester nanofibers was heterogeneous and the release behaviour of proteins was poorly controlled (Lai et al., 2014). All of these flaws greatly impaired the protein delivery performance of the particle-loaded polyester nanofibers.

In this study, we intended to rationally design the particle preloading method to avoid these deficiencies and improve the protein delivery performance of polyester nanofibers. As shown in Fig. 1, to guarantee the activity retention of proteins in the preloading step, we used positively charged chitosan nanoparticles (CNPs) as carriers to adsorb negatively charged proteins in mild conditions. The nanoparticles were then dispersed in a solution of poly(lactic acid) (PLA) in 1,1,1,3,3,3,-hexafluoro-2-propanol (HFIP) and subjected to electrospinning. The nanoparticle matrix could prevent proteins from losing bioactivity during the electrospinning process. The nanoparticles' small size and similarity of polarity to the electrospinning solution could enable their homogeneous dispersion in the solution, the subsequent homogeneous distribution within the electrospun polyester nanofibers, and the sustained release of proteins. The morphology, size, and wettability of the nanofibers along with the distribution of the nanoparticles within the polyester nanofibers were characterized in detail by a scanning electron microscope (SEM), contact angle measurements and a laser scanning confocal microscope (LSCM). The release behaviour and bioactivity of proteins were examined by in vitro incubation and a cell proliferation assay, respectively. Cell adhesion and spreading on the polyester nanofibers were examined with cell culture experiments, as well as any cytotoxic effects. The application potential of the protein delivering polyester nanofibers was evaluated with in vivo wound healing experiments.

2. Materials and methods

2.1. Materials

Chitosan (Mw ≈ 600 kDa, degree of deacetylation = 92.9%) was purchased from Sinopharm Chemical Reagents (Beijing, China). PLA (Mn = 265 kDa)was kindly gifted from Changchun Sinobiomaterials Co. Ltd. (Changchun, China). Bovine serum albumin fraction V (BSA), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT), and the BCA protein assay kit were purchased from Dingguo Biotech (Beijing, China). Epidermal growth factor (EGF) was supplied by PeproTech (New Jersey, US). Sodium tripolyphosphate (TPP) and HFIP were purchased from Aladdin Reagents (Shanghai, China). Streptozotocin (STZ) was purchased from Meilune Biotech. (Dalian, China). All other chemical reagents were purchased from Xilong Chemicals (Guangzhou, China) and used as received. Deionized water was used at all times.

2.2. Methods

2.2.1. Preloading protein on chitosan nanoparticles (CNPs)

Proteins were first loaded onto CNPs by the dipping method. First, chitosan nanoparticles were fabricated by the ionic gelation method and purified by centrifugation and repeated washing as previously reported (Hou et al., 2012). Then, a certain amount of model protein BSA or EGF was added to the chitosan nanoparticle suspension (20 mg/mL in water). The mixture was incubated at 4 °C for 48 h to allow sufficient interactions between proteins and chitosan nanoparticles. The protein-loaded CNPs were collected by centrifugation (11,000 rpm, 30 min) and rinsed twice with deionized water. The supernatant was used to collect a UV absorbance measurement (280 nm) with a UV–vis spectrometer (Lambda 900, Perkin Elmer). The drug encapsulation efficiency

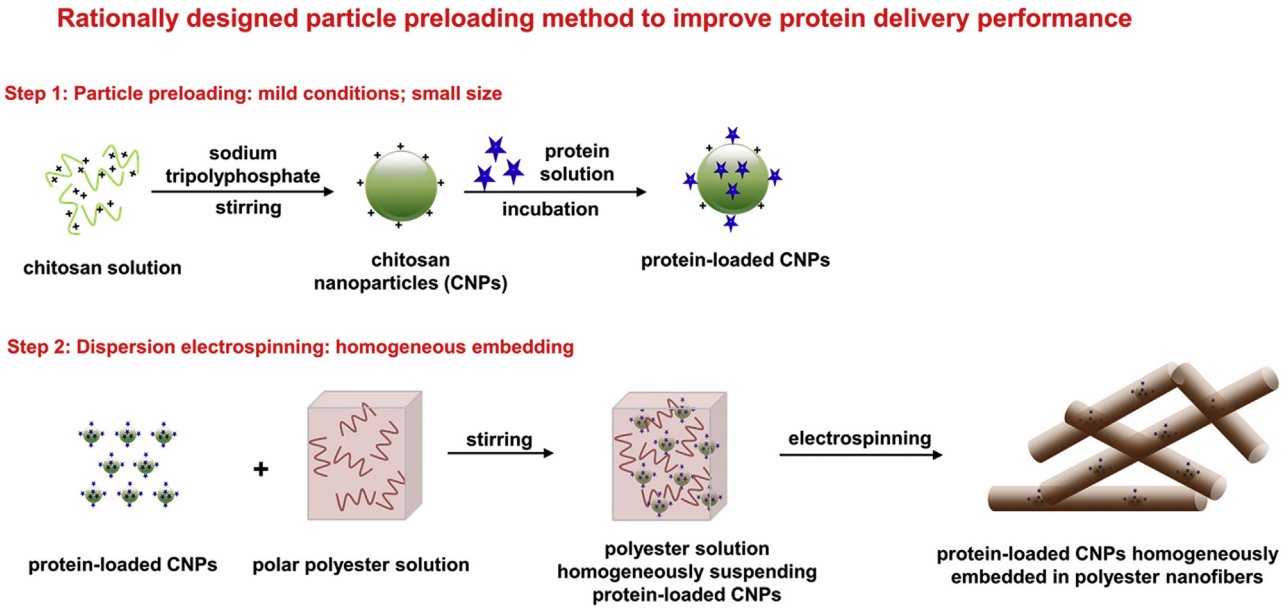

Fig. 1. Schematic illustration of the rationally designed particle preloading method to improve protein delivery performance of electrospun polyester nanofibers.