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Controlled release and antibacterial activity of antibiotic-loaded electrospun halloysite/poly(lactic-co-glycolic acid) composite nanofibers

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ABSTRACT

Fabrication of nanofiber-based drug delivery system with controlled release property is of general interest in biomedical sciences. In this study, we prepared an antibiotic drug tetracycline hydrochloride (TCH)-loaded halloysite nanotubes/poly(lactic-co-glycolic acid) composite nanofibers (TCH/HNTs/PLGA), and evaluated the drug release and antibacterial activity of this drug delivery system. The structure, morphology, and mechanical properties of the formed electrospun TCH/HNTs/PLGA composite nanofibrous mats were characterized using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy, and tensile testing. We show that the incorporation of TCH-loaded HNTs within the PLGA nanofibers is able to improve the tensile strength and maintain the three-dimensional structure of the nanofibrous mats. *In vitro* viability assay and SEM morphology observation of mouse fibroblast cells cultured onto the fibrous scaffolds demonstrate that the developed TCH/HNTs/PLGA composite nanofibers are cytocompatible. More importantly, the TCH/HNTs/PLGA composite nanofibers are able to release the antibacterial drug TCH in a sustained manner for 42 days and display antimicrobial activity solely associated with the encapsulated TCH drug. With the improved mechanical durability, sustained drug release profile, good cytocompatibility, and non-compromised therapeutic efficacy, the developed composite electrospun nanofibrous drug delivery system may be used as therapeutic scaffold materials for tissue engineering and drug delivery applications.

1. Introduction

Electrospinning is a versatile polymer processing technique to fabricate engineered scaffolds with micro to nanoscale topography and high porosity mimic to the natural extracellular matrix (ECM) [1,2]. It offers great flexibility in terms of material selection for drug delivery applications, and a rich variety of drugs have been physically or chemically formulated within electrospun nanofibers or on their surfaces, such as antibiotics [3–7], anticancer drugs [8,9], proteins [10,11], and DNA [12–14]. Meanwhile, the drug release mechanism is associated with polymer degradation and diffusion pathway, and the release profile of drug from electrospun nanofibers may be tuned by the polymer composition and fiber morphology [15]. More importantly, drug-loaded electrospun nanofibrous mats possess better site-specificity and lower overall medicinal dosages than conventional drug delivery system. Therefore, drug-loaded electrospun nanofibers display obvious advantages when applied as tissue engineering scaffolds, wound healing materials, abdominal anti-adhesions after surgical procedure, or in post-operative local chemotherapy [16–18].

So far, a number of different drug loading methods have been developed to prepare drug-loaded nanofibers [19–26]. Typically, drug-loaded nanofibers can be formed easily and directly by electrospinning polymer blends/mixtures with drugs. However, the burst release effect at the initial period is often unavoidable. Therefore, coaxial electrospinning and emulsion electrospinning approaches have been utilized to form core-shell nanofibers with drug enclosed in the core region of the fibers [21–23]. In this case, the diffusion rate of the therapeutic agent will be fairly stable, and the drug could be released in a sustained manner. Nevertheless, the stringent specification of the electrospinning equipment in the coaxial method and the poor biocompatibility of emulsifiers in the emulsion method limit their biomedical applications. More recently, Kissel et al. fabricated 'nano in nano' composites

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for drug delivery, which consist of biodegradable drug-loaded polymer nanoparticles incorporated into polymer nanofibers. The formed composite nanofibers can efficiently modulate drug delivery, but an extra step to prepare drug-loaded polymer nanoparticles is required prior to the fabrication of the nanofibers [24]. In our previous work, we reported a novel electrospun composite nanofiber-based drug delivery system [25,26]. Inorganic nanotubes (halloysite nanotubes, HNTs) were used to encapsulate a model drug tetracycline hydrochloride (TCH) first, and then the TCH-loaded nanotubes were mixed with biocompatible polymer (poly(lactic-co-glycolic acid), PLGA) solution for subsequent electrospinning to form composite drug-loaded nanofibers. Both the HNTs and PLGA fiber matrix can act as drug delivery carriers, and this double-container drug delivery system has been proven to be able to reduce the drug release rate and significantly suppress the initial burst release. However, the antibacterial efficacy of the TCH-loaded nanofibers has not been explored and the drug loading process needs to be further optimized.

In this study, with the continuation of our previous work, we fabricated HNTs/PLGA composite nanofibers loaded with an antibiotic drug, TCH, and evaluated the drug release and antibacterial activity of this drug delivery system. Firstly, TCH was encapsulated within HNTs, and then the drug-loaded composite nanofibers (TCH/HNTs/PLGA) were fabricated by electrospinning the mixture solution of drug-loaded HNTs and PLGA. As a control, the conventional type of TCH-loaded PLGA nanofibers (TCH/PLGA) was also prepared by electrospinning mixture solution of TCH and PLGA. The formed nanofibers were characterized using scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and mechanical testing. The cytocompatibility of electrospun nanofibrous scaffold was investigated through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay and SEM morphology observation of the mouse fibroblasts cultured onto the drug-loaded nanofibrous scaffolds. The drug release kinetics and the *in vitro* antimicrobial activities of the drug-loaded composite nanofibers were also investigated. To our knowledge, this is the first report related to the antibacterial activity study of the electrospun HNTs-doped composite drug delivery system.

2. Experimental

2.1. Materials

PLGA (Mw = 81,000 g/mol) with a lactic acid/glycolic acid ratio of 50:50 and TCH (purity > 95%) were purchased from Jinan Jianbao Kaiyuan Biotechnology Co., Ltd. (China) and Sigma–Aldrich, respectively. Halloysite was a gift from Zhengzhou Jinyangguang China Clays Co., Ltd. (China). Tetrahydrofuran (THF), N,N-dimethylformamide (DMF), and beef extract were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). *Staphylococcus aureus* (*S. aureus*) was purchased from Shanghai Fuzong Biotechnology Development Co., Ltd (China). Peptone was from Beijing Aoboxing Biotechnology Co., Ltd. (China), and agar was purchased from Beijing Biodev-tech Scientific & Technical Co., Ltd (China). Mouse fibroblasts were obtained from Institute of Biochemistry and Cell Biology (the Chinese Academy of Sciences, Shanghai, China). Unless otherwise specifically explained, all cell culture medium and reagents were purchased from Hangzhou Jinuo Biomedical Technology Co., Ltd. (China). Water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with resistivity higher than 18 MΩ cm.

2.2. Preparation of drug-loaded composite nanofibers

TCH was loaded within HNTs according to the procedure described in our previous report with slight modification [25]. Instead dissolving TCH powder in phosphate buffered saline (PBS buffer, pH 7.4) at 80 °C for 20 min reported in our previous study [25], in this study TCH was dissolved in water at 80 °C for 20 min. Detailed experimental procedure can be found in Supporting information. The drug loading efficiency using HNTs was optimized by changing the weight ratio between TCH and HNTs from 1:2, 1:1, 1.5:1 to 2:1, and the optimized weight ratio was confirmed at 1.5:1. The loading efficiency was calculated according to the original concentration of TCH and the concentration of unloaded TCH quantified using a Lambda 25 UV-vis spectrophotometer (Perkin Elmer, USA) at the monitoring wavelength of 270 nm. The loading percentage of TCH onto or into HNTs was also calculated by dividing the mass of the encapsulated TCH by the mass of the TCH-loaded HNTs powder.

PLGA with an optimized concentration (25%, w/v) was dissolved in a mixed solvent of THF/DMF (v/v = 3:1). HNTs (3 wt% relative to PLGA) and TCH-loaded HNTs (1 and 2% of TCH relative to PLGA) were blended with PLGA solution for subsequent electrospinning. Drug-loaded TCH/HNTs/PLGA nanofibers with different percentages of TCH (1 and 2%) were denoted to TH-1/PLGA and TH-2/PLGA, respectively. Moreover, a mixture solution of TCH and PLGA was also prepared to form the control fibers of TCH/PLGA without HNTs. TCH (20 mg/mL) solution was first prepared by dissolving the TCH powder in hexafluoroisopropanol (HFIP). Then a measured volume of TCH (1 wt% relative to PLGA) solution was added into PLGA solution with continuous stirring for 1 h until a clear and homogeneous solution was obtained before electrospinning. The electrospinning system and procedure used in this study were similar to that used in our previous work [25,26]. Details can be found in Supporting information.

2.3. Characterization techniques

Morphologies of the PLGA, HNTs/PLGA, TCH/HNTs/PLGA, and TCH/PLGA nanofibers were observed using SEM (JEOL JSM-5600LV, Japan) with an accelerating voltage of 15 kV. Before SEM observations, the samples were sputter-coated with gold films with a thickness of 10 nm. The diameters of the electrospun fibers were analyzed using ImageJ 1.40G software (http://rsb.info.nih.gov/ij/download.html, National Institutes of Health, USA). At least 200 nanofibers at different images were analyzed for each sample.

The mechanical properties of the electrospun fibrous mats were examined using a materials testing machine (H5K-S, Hounsfield, UK) with an elongation speed of 10 mm/min at 20 °C and a relative humidity of 63%. Before measurements, five strips from different sites of each nanofibrous sample were chosen for the tensile test, and specimens were cut into rectangular pieces with width × length of 10 mm × 50 mm. The thickness of each specimen was measured with a micrometer before tensile test. Then, the two ends of sample were gripped in the top and bottom chucks, respectively, and the gauge of two chucks connected to testing machine was fixed at 30 mm. The tensile testing was performed with a load cell of 10 N. Stress and strain were calculated through the following equations:

$$\sigma\,(\mathrm{MPa}) = \frac{P\,(\mathrm{N})}{w\,(\mathrm{mm}) \times d\,(\mathrm{mm})} \quad (1)$$

$$\varepsilon = \frac{l}{l_0} \times 100\% \quad (2)$$

where σ, ε, P, w, d, l, and l_0 stand for stress, strain, load, mat width, mat length, extension length, and gauge length, respectively.