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Characterization and antibacterial activity of amoxicillin-loaded electrospun nano-hydroxyapatite/poly(lactic-co-glycolic acid) composite nanofibers

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ABSTRACT

We report a facile approach to fabricating electrospun drug-loaded organic/inorganic hybrid nanofibrous system for antibacterial applications. In this study, nano-hydroxyapatite (n-HA) particles loaded with a model drug, amoxicillin (AMX) were dispersed into poly(lactic-co-glycolic acid) (PLGA) solution to form electrospun hybrid nanofibers. The loading of AMX onto n-HA surfaces (AMX/n-HA) and the formation of AMX/n-HA/PLGA composite nanofibers were characterized using different techniques. We show that AMX can be successfully adsorbed onto the n-HA surface and the formed AMX/n-HA/PLGA composite nanofibers have a uniform and smooth morphology with improved mechanical durability. Cell viability assay and cell morphology observation reveal that the formed AMX/n-HA/PLGA composite nanofibers are cytocompatible. Importantly, the loaded AMX within the n-HA/PLGA hybrid nanofibers shows a sustained release profile and a non-compromised activity to inhibit the growth of a model bacterium, *Staphylococcus aureus*. With the significantly reduced burst-release profile, good cytocompatibility, improved mechanical durability, as well as the remained antibacterial activity, the developed AMX/n-HA/PLGA composite nanofibers should find various potential applications in the fields of tissue engineering and pharmaceutical science.

1. Introduction

Electrospinning, a technique producing ultrafine fibers with diameters ranging from tens of nanometers to several microns, has attracted much attention due to its versatility and potential for applications in diverse fields [1–4]. The features of electrospun nanofibers with high specific surface area, high porosity, and three-dimensional (3D) reticulate structure quite mimic the natural extracellular matrix (ECM) [5], affording them with a wide range of biomedical applications including tissue engineering [6], wound dressing [7], biosensors [8], and drug delivery [5,9–12]. In particular for drug delivery applications, conventional [9,13], emulsion [14,15] and coaxial [12] electrospinning techniques have been used to fabricate nanofibers for drug encapsulation and release.

Conventional single fluid electrospinning method allows direct integration of drug molecules within nanofibers by simply electrospinning the drug/polymer mixture solution or post-adsorption of drugs onto/within the nanofibers [11,13,16,17]. However, a burst release often happens, which is not desirable in most of the cases. The techniques of emulsion and coaxial electrospinning used for drug delivery applications are able to alleviate the burst release of the encapsulated drug to some extent [15,18,19]. In both methods, the drugs are able to be incorporated into the core region of the nanofibers to form a "core–sheath" structure, in which the outer polymer shell can act as an additional barrier to control the drug release profile [20]. However, there are still some issues and challenges in the emulsion and coaxial electrospinning techniques. The coaxial electrospinning may need substantial optimization of the electrospinning parameters, and the emulsifier used in emulsion electrospinning may be cytotoxic. In another aspect, the electrospun nanofibers composed of polymer or polymer blends often suffer a problem with mechanical durability, which is not desirable for practical biomedical applications. Therefore, development of other nanofiber systems that possess mitigated burst release of the encapsulated drug and/or improved mechanical durability still remains a great challenge.

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In our previous work, we have shown that drug-loaded halloysite nanotubes (HNTs), a naturally occurring clay material, can be incorporated within poly(lactic-co-glycolic acid) (PLGA) nanofibers by simply electrospinning the mixture solution of PLGA and HNTs-drug particles [5]. The thus formed hybrid nanofibers afford the drug with significantly decreased burst release profile, and simultaneously the incorporation of HNTs greatly improves the mechanical durability of the nanofibers [5,21–23]. The incorporated HNTs within the nanofibers play two roles: firstly, the HNTs themselves are a kind of drug carrier, which allows drug molecules to be encapsulated within the lumen of the HNTs; secondly, the presence of HNTs is able to significantly improve the mechanical durability of the nanofibers. The previous success leads us to hypothesize that other inorganic nanoparticles having a capability to encapsulate drug molecules may also be incorporated within polymer nanofibers to alleviate the burst release of drug molecules and to improve the mechanical durability of the fibers, thereby providing a vast range of opportunities for various biomedical applications.

Nano-hydroxyapatite (n-HA) has been considered as an ideal inorganic drug carrier due to its high surface area to volume ratio, high surface activity, good biocompatibility, and strong ability to absorb a variety of chemical species [24]. However, the weak interaction between the drug molecules and the n-HA particles often leads to an initial burst release of the drugs from the formed n-HA/drug nanocomplex [25]. Therefore, it is quite reasonable to design a hybrid n-HA-incorporated polymer nanofiber system, where both polymer nanofibers and the n-HA are containers and barriers of drug molecules, affording the drug with a sustained release profile. Likewise, the presence of n-HA within the polymer nanofibers is also expected to share a portion of load applied on the nanofibrous mats, improving the mechanical durability of the fiber system.

In this present work, we attempted to develop a facile approach to fabricating n-HA-doped PLGA nanofibers via electrospinning for drug encapsulation and release. A model drug, amoxicillin (AMX) was first loaded onto the n-HA surface via physical adsorption. Then the AMX-loaded n-HA particles were mixed with PLGA solution for subsequent formation of electrospun AMX/n-HA/PLGA composite nanofibers (Scheme 1a). The loading of AMX onto n-HA (n-HA/AMX) and the formation of AMX/n-HA/PLGA composite nanofibers were characterized using different techniques. The release kinetics of AMX from the composite AMX/n-HA/PLGA nanofibers was investigated using UV–Vis spectroscopy and compared with AMX/n-HA particles and AMX/PLGA nanofibers. The antimicrobial activity of the AMX/n-HA/PLGA nanofibers was investigated using *Staphylococcus aureus* (*S. aureus*) as a model bacterium both in liquid and on solid medium. The cytocompatibility of AMX/n-HA/PLGA nanofibers was investigated through in vitro 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay and microscopic imaging of cells cultured onto the composite fibrous scaffolds.

2. Experimental section

2.1. Materials

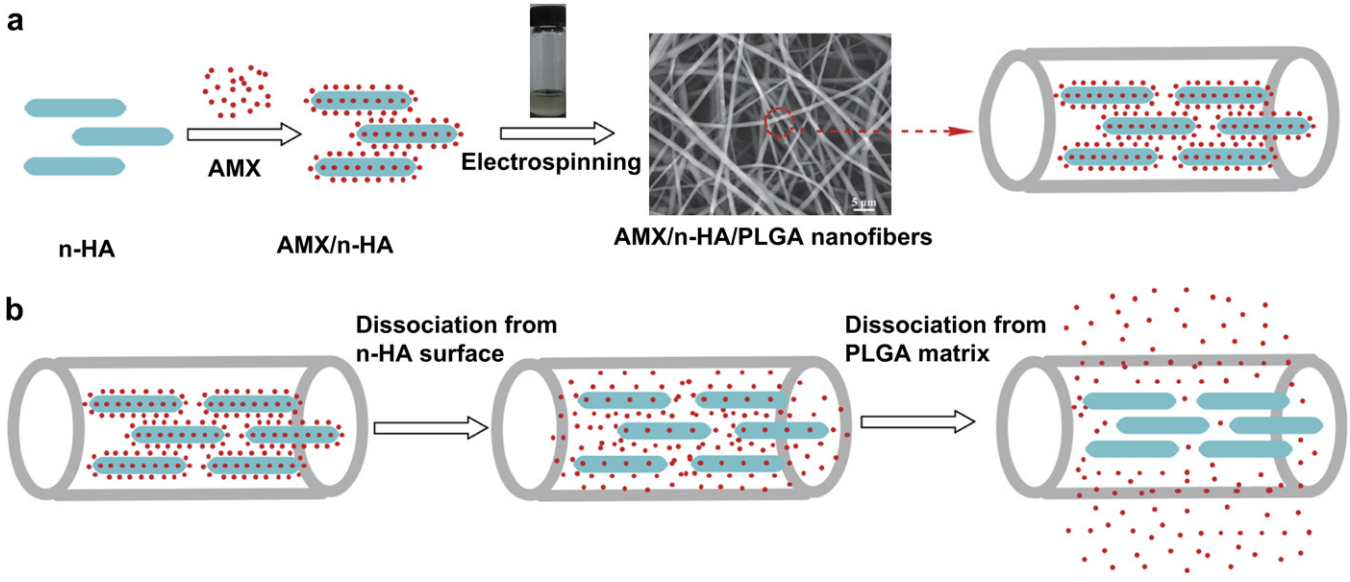
PLGA (MW = 81 000 g/mol) with a lactic acid/glycolic acid ratio of 50:50 and AMX (purity > 95%) were purchased from Jinan Daigang Biotechnology Co., Ltd. (China) and Shanghai Yuanye Biotechnology Co., Ltd. (China), respectively. n-HA was obtained from Aladdin Chemical Reagent Co., Ltd. (China). *S. aureus* was purchased from Shanghai Fuzhong Biotechnology Development Co., Ltd. (China). Luria-Bertani (LB)-medium was acquired from Sangon Biotech Co., Ltd. (Shanghai, China). Tetrahydrofuran (THF) and N,N-dimethylformamide (DMF) were from Sinopharm Chemical Reagent Co., Ltd. (China). Mouse fibroblasts (L929) were obtained from Institute of Biochemistry and Cell Biology (the Chinese Academy of Sciences, Shanghai, China). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Hangzhou Jinuo Biomedical Technology (Hangzhou, China). Fluorescein diacetate (FDA) was purchased from Sigma. All chemicals were used as received. 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. Loading of AMX onto the surface of n-HA

An aqueous solution of AMX (10 mL, 4 mg/mL) was added dropwise into an aqueous suspension of sieved n-HA (10 mL, 2 mg/mL). The mixture was then vigorously stirred for 24 h at room temperature to form AMX-loaded n-HA (AMX/n-HA). The formed AMX/n-HA nanohybrid was separated by centrifugation (5000 rpm, 3 min) and washed with water three times to remove the excess free AMX non-adsorbed onto the n-HA surface. The AMX concentration in the supernatant was analyzed using Lambda 25 UV–Vis spectrophotometer (Perkin Elmer, USA) at 228 nm using a standard AMX concentration-absorbance calibration curve and the AMX loading percentage was calculated by Eq. (1):

$$\text{Loading percentage} = M_t/(M_t + M_o) \times 100\% \quad (1)$$

where M_t and M_o stands for the mass of adsorbed AMX and the n-HA carrier, respectively. Finally, the AMX/n-HA was lyophilized, ground down, and sieved to have a uniform size for subsequent electrospinning process. The drug loading



Scheme 1. Schematic illustration of the encapsulation (a) and release pathways (b) of AMX within n-HA-doped PLGA nanofibers.