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Silver-functionalized Polyurethane Composite Nanofibers for Controlled Release and Antibacterial Application*

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Abstract

In this study, we report a facile approach to develop composite nanofibrous mat for tissue engineering application. Montmorillonite (MMT) nanoparticles were first used to load an antipyretic analgesic drug, aspirin (ASP). The ASP-loaded MMT nanohybrids were mixed with polyurethane (PU) for subsequent electrospinning to form drug-loaded PU/MMT/ASP composite nanofibrous mats. Then electrospun PU/MMT/ASP nanofibers were assembled with a bilayer of polyacrylic acid (PAA) and poly(ethylene imine) (PEI) through electrostatic interaction. Silver nanoparticles have been immobilized onto nanofibrous mats by in situ complexation and chemical reduction of $AgNO_3$ solution to form PU/MMT/ASP/Ag nanofibrous mats. The PU/MMT/ASP/Ag composite nanofibrous mats were systematically characterized using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy, and mechanical testing. In vitro drug release showed that this composite nanofibrous drug delivery system can effectively mitigate the burst release of the drug and the introduction of MMT can improve the tensile stress property. Further the antibacterial properties and cytotoxicity evaluation of these mats demonstrate that the PU/MMT/ASP/Ag has a reasonable activity toward the growth inhibition of model bacterium Staphylococcus aureus, and the PU/MMT/ASP/Ag nanofibers display good cytocompatibility. In view of its sustained release profile and excellent biocompatibility, this double-loaded drug delivery system may have great prospect in tissue engineering.

Keywords: Electrospinning; Polyurethane; Montmorillonite; Aspirin; Silver Nanoparticles

1 Introduction

It is known that tissue engineering includes cells, materials and biochemical elements for the construction of a new tissue to replace the impaired one [1, 2]. The electrospinning technique is considered to be a versatile approach to generate sequential nanofibers for this purpose, because of the superior properties of electrospun nanofibers such as large specific area, high area-to-volume ratio and high porosity. In particular, the three-dimensional network structure of electrospun nanofibrous mats that can mimic human extracellular matrix (ECM) has aroused great interest

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of scientific research workers [3, 4]. Many materials such as biodegradable, non-degradable, and natural materials have been used to produce electrospun nanofibers, the characteristics of which like diameter, appearance and mechanical properties can be regulated to fit for cells adhesion and proliferation [5-7].

Recently, nanofibers have been widely studied as drug delivery system on account of their advanced properties [8]. A majority of drug-loaded electrospun nanofibers were fabricated from blends [9, 10] in core-shell method [11] or separate-spinneret manner [12]. However, although this method enables easy incorporation of drug molecules into the nanofibers, a burst release always occurs which is undesirable in many cases [13, 14]. For instance, Verreck and co-worker incorporated poorly water-soluble drug, itraconazole and ketanserin drugs into electrospun segmented polyurethane nanofibers for potential drug administration and wound healing. Drug release study showed that in the first 4 h, the content of model drug ketanserin released from PU nanofibrous mats reached 5 $\mu g/cm^2$, 50% of loaded drug has been released out compared to 10 $\mu g/cm^2$ within 24 h, suggesting an obvious burst release phenomenon [15]. Thus, developing nanofibrous drug delivery system that can mitigate the burst release of the model drugs is still a challenge.

Montmorillonite (MMT), a kind of non-toxic and layered silicate material has been frequently used in biomedical field as a drug carrier because of its high internal surface area, excellent dispersive performance and biocompatibility [16]. Moreover, negatively charged MMT has good swelling property in the presence of water and hydrophilic solvents because of which the positively charged bioactive compounds can be intercalated into the interlayer spaces by electrostatic interaction [17, 18]. In view of the advantages of MMT, we hypothesize that incorporating drug-loaded MMT into electrospun nanofbers might be effective to reduce the bust release of model drug. In this study, we choose PU, a widely used polymer in the biomedical field owing to its well physical, chemical and biological properties, especially excellent antimicrobial activity and cytocompatibility [19] to produce electrospun nanofibers. The anti-inflammatory drug aspirin (ASP) was chose as a model to be incorporated within MMT firstly, then ASP-loaded MMT was blended with PU solution to form a PU/MMT/ASP nanofibers by electrospinning technology. Further, silver nanoparticles, which is the most powerful antimicrobial that exhibits a strong cytotoxicity toward a broad range of microorganisms, and a remarkably low human toxicity compared to other heavy metal ions [20, 21], has been deposited into PU/MMT/ASP nanofibrous mats by chemical reduction of silver nitrate solution to form PU/MMT/ASP/Ag nanofibrous mats with antibacterial property.

The developed PU/MMT/ASP/Ag composite nanofibrous mats were systematically characterized using Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FTIR) spectroscopy and mechanical testing. The drug release kinetics of the PU/MMT/ASP nanofibrous mat was monitored using ultraviolet-visible (UV-Vis) spectrometer. The antibacterial property of nanofibers with different compositions was investigated using *Staphylococcus aureus (S.aureus)* as a model bacterium by the disk-diffusion method. Finally, the cytocompatibility of PU/MMT/ASP/Ag composite nanofibers was detected via Rezasurin assay to evaluate the cell viability of fibrous blast cell L929 cultured on the nanofiber scaffolds.

2 Materials and Methods

Polyurethane (PU) was purchased from Dow Chemical Co., Ltd. Acetone and dimethyl formamide (DMF) and $AgNO_3$ were bought from Sinopharm Chemical Reagent Co., Ltd. Montmorillonite (MMT) was purchased from Zhejiang Feng Hong New Material Co., Ltd. Aspirin