Journal of Fiber Bioengineering and Informatics 10:2 (2017) 105–116
doi:10.3993/jfbim00266

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

Qing-Yan Peng*, Hao Wang, Shi-Li Xiao*

School of Textile Science and Engineering, Wuhan Textile University, Wuhan, Hubei 430000, China

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

*Project financially supported by the National Natural Science Foundation of China (51403165) and the Education Commission of Hubei Province (D20161602).

*Corresponding author.
Email addresses: 15629184851@163.com (Qing-Yan Peng), xshili@yahoo.com (Shi-Li Xiao).

1940–8676 / Copyright © 2017 Textile Bioengineering and Informatics Society
Jun. 2017

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

(ASP) (purity $\geq$ 99%), polyacrylic acid (PAA) and poly(ethylene imine) (PEI) and sodium borohydride ($NaBH_4$) were from Aladdin Industrial Corporation (Shanghai). Staphylococcus aureus (S.aureus) was obtained from Shanghai Fuzong Biotechnology Development Co., Ltd (China). Peptone was from Beijing Aoboxing Biotechnology Co., Ltd (China). Agar was purchased from Beijing Solarbio Science & Technology Co., Ltd (China). Cells L929 was purchased from Wuhan Servicebio Biotechnology Co., Ltd (China).

2.1 Preparation of the Drug-loaded MMT Nanohybrids

ASP was dissolved into phosphate buffered solution (PBS) (pH = 7.4) to acquire ASP aqueous solution (0.25 mg/mL) at room temperature. After that, MMT powder was dispersed into ASP solution with different concentrations, namely 0.25, 0.5, 1, 2 and 4 mg/mL, respectively, to get serious of MMT/ASP suspensions. Subsequently, the MMT/ASP suspensions were vigorously stirred for 12 h to allow sufficient interaction between ASP and MMT. The mixture were then centrifuged under 6000 rpm for 10 min to remove the free ASP and then rinsed with PBS for 3 times. The supernatants after 4 times centrifugation were collected together. The ASP encapsulation and loading efficiencies were determined by measuring the absorbance at 274 nm using a UV-756PC UV-visible Spectrometer and a standard curve for ASP (prepared using ASP solution of different known concentration). The prepared MMT/ASP nanohybrids were lyophilized and stored in desiccator before use. The drug loading efficiency can be calculated from the following equation [22]:

$$Loading\ efficiency = (M_1 - M_0)/M \times 100\% \quad (1)$$

where M_1 and M_0 stand for the mass of initial ASP used for encapsulation and the free ASP, respectively, and M stands for the total mass of ASP and MMT.

2.2 Preparation of the PU/MMT/ASP/Ag Composite Nanofibers

PU was dissolved in a mixture solvent of acetone/DMF (v/v = 1:1) with an optimized concentration of 15% (w/v). Subsequently, MMT/ASP nanohybrids (with the optimized proportion, 2% w/v) were dispersed in PU solution under magnetic stirring for 12 h for preparing PU/MMT/ASP nanofibrous mats. Pure PU and PU with MMT nanofibrous mats were also prepared as a control. The electrospinning process was carried out under ambient condition with a fixed electrical potential of 20 kV, a collector distance of 20 cm, and a feeding rate of 0.5 mL/h. The prepared electrospun nanofibrous mats with different compositions were taken off from the collector and vacuum-dried for 12 h to remove the residual organic solvent before use. Subsequently, the formed PU/MMT/ASP nanofibrous mat was then assembled with the PAA/PEI bilayer through electrostatic interaction [23, 24]. That is, 1 mg/mL aqueous solutions of PAA and PEI were separately prepared in 30 mg/mL NaCl solution, a square of PU/MMT/ASP nanofibrous mat (5 cm $\times$ 5 cm) was exposed to PAA solution for 10 min, rinsed with water 3 times (2 min each time). The PAA-assembled PU/MMT/ASP mats was then exposed to PEI solution for 10 min and rinsed with water following the above process. To incorporate Ag into the PAA/PEI-assembled PU/MMT/ASP nanofibrous mat, 0.1 M $AgNO_3$ was prepared and the PAA/PEI-assembled PU/MMT/ASP nanofibrous mats were immersed to it under gentle shaking for 2 h. After that, the formed nanofibrous mats were thoroughly rinsed with water and dried in a vacuum oven for at least 12 h to remove the additional liquid. Then 1 M $NaBH_4$ was added evenly onto

the dried nanofibrous mats. PAA/PEI-assembled PU/MMT/ASP nanofibrous mats without Ag were also prepared as a control.

2.3 Characterization

The PU and PU/MMT/ASP nanofibrous mats were characterized using FTIR spectroscopy. The spectra were recorded using a Nicolet Nexus 470 FTIR spectrometer (Thermo Nicolet Corporation, United States) in a wavenumber range of 4000-400 cm^{-1} at ambient conditions. The surface morphology of PU, PU/ASP, PU/MMT, PU/MMT/ASP and PU/MMT/ASP/Ag composites nanofibers was detected with scanning electron microscope (SEM) (HITACHI UHR FE-SEM SU1510) at an accelerating voltage of 15 kV. Before detection, each specimen was spray-coated with a 15 nm thick gold film. Fiber diameter was measured using ImageJ 1.49 (http://rsb.info.nih.gov/ij/download.html), at least 200 nanofibers from SEM images were randomly selected and analyzed. Tensile test of the electrospun nanofibrous mats were performed by electronic material testing machine (INSTRON3300, UK) at constant temperature (20 °C) and humidity (63%) with a cross-head speed of 1 cm/min. Specimens were cut into rectangular pieces with width × length= 1 cm × 5 cm and were gripped in the top and bottom collets 2 cm apart. The tensile testing was performed with a loading of 10 N. According to the collected data, the stress-strain curves of the samples can be calculated through mathematical conversions [25]:

$$\delta(MPa) = \frac{f(N)}{w(mm) \times h(mm)} \tag{2}$$

$$\varepsilon(\%) = \frac{L(mm)}{g(mm)} \times 100\% \tag{3}$$

where f is the breaking force of sample; w and h are the width and thickness of the sample, respectively; l is the breaking length and g is the distance between top and bottom collets.

2.4 In Vitro Drug Release Analysis

In vitro release of ASP from PU/ASP and PU/MMT/ASP (the content of ASP in nanofibrous mat was 2%) nanofibrous mats was evaluated using ultraviolet-visible (UV-Vis) spectroscopy (UV-756PC). 60 mg of each specimen was put into 20 mL vial containing 5 mL PBS (pH = 7.4) solution with gently shaking in an oscillator (SKY-2102C) at 37 °C. At different time intervals, 2 mL of solution was taken out and replenished with an equal volume of corresponding buffer solution. The optical density value was measured using an UV-Vis spectrometer at 274 nm. Thus, the concentration of released ASP at different time interval can be calculated using standard curve of the concentration vs. absorption. Simultaneously, the accumulated release concentration can also be obtained. All the samples were analyzed in triplicate.

2.5 Cytotoxicity Evaluation

Cytotoxicity of electrospun PU, PU/ASP, PU/MMT/ASP, and PU/MMT/ASP/Ag nanofibrous mats were evaluated using L929 cells directly cultured on the samples. Glass coverslips were covered with nanofibrous mats and placed in the bottom of the wells of 24-well culture plates.

For sterilization, the mats were initially left under UV radiation overnight, followed by soaking with 75% ethanol solution for 1.5 h and ultimately washed three times with PBS solution. The electrospun nanofibrous mats were then soaked in culture medium overnight prior to cell seeding. Cells were seeded on the top of the mats at a density of 2×10^4 cells per well, and cultured in 1 mL DMEM cell culture medium (Gibco) containing 1% Antibiotic-Antimycotic solution (AA, Gibco) and 10% fetal bovine serum (FBS, Gibco) in a humidified atmosphere with 5% CO_2 at 37 °C. After 24 h, the medium was changed by fresh medium containing 10% (v/v) of a rezasurin solution, followed by incubation for another 3 h. Subsequently, 100 Ul of resultant medium was transferred to an opaque 96-well plate and the resorufin fluorescence (λ_{ex} = 530 nm, λ_{em} = 590 nm) was measured in a microplate reader (Perkin Elmer, Victor^3 1420). The cell morphology was observed by SEM (JEOL JSM-5600LV, Japan) with an acceleration voltage of 10 kV. Before this, cell cultured on each sample were rinsed 3 times with PBS solution to remove non-adherent cells. Then each sample was fixed with 2.5% glutaraldehyde at 4 °C for 2 h, followed by dehydrating through a series of gradient ethanol, and air dried overnight. Before SEM observation, samples were sputter coated with a 10 nm thick gold film.

2.6 Antibacterial Activity

To evaluate the antibacterial activity of PU, PU/MMT/ASP, and PU/MMT/ASP/Ag composite nanofibers, the Kirbydisc diffusion method was used and this test was performed on *S. Aureus* as model bacterium. During the test, specimens of different formulations were cut into square discs with 2 cm × 2 cm and sterilized under ultraviolet lamp (ZW30S25W-Z894, Guangdong Cnlight) for 2 h. The *S. aureus* were seeded on agar plates with a sterilized cotton stick and then the specimens were placed gently on the surface of the agar plate using a sterilized forceps. During this, the disks can not to be moved once it contacts with the agar surface. The plates were then immediately placed in the incubator at 37 °C for 24 h. Finally, the plates were examined and the diameters of the zones were measured for complete inhibition, including the diameter of the disk. The tests were carried out in triplicate to confirm reproducibility.

2.7 Statistics

All statistical analysis was performed using the IBM SPSS Statistics 20 software (IBM Inc., Armonk). Results are reported as the mean ± standard deviation. One-way ANOVA with Tukey Post Hoc test were used to assess the statistical differences between the group means.

3 Results and Discussion

3.1 Preparation of ASP/MMT Nanohybrid

Montmorillonite (MMT) is a layered aluminum silicate with exchangeable cations and reactive-OH groups on the surface. It is one of the most widely used layered silicates because its lamellar elements display high in-plane strength, stiffness, high aspect ratio, and high surface areas in particular [26, 27]. Recent studies have shown that incorporating bioactive cargos or drugs into silicate clay-doped polymers could be a promising way to develop functional tissue engineering

materials [28]. Herein, we load antipyretic analgesic drug ASP onto MMT nanoparticles to produce nanohybrids for controlled drug release materials. Fig. 1 shows the loading efficiency of ASP with a function of MMT concentration, where the concentration of ASP solution was fixed at 0.25 mg/mL. As we can see, when the concentration of MMT was lower than 2 mg/mL, the loading efficiency of ASP on MMT nanoparticles increased with the increasing of the MMT solution concentration. The loading efficiency of ASP on MMT nanoparticles reached up to 51.7% when the concentration of MMT was 2 mg/mL. However, further increasing the concentration of MMT solution resulted in a decreased loading efficiency of ASP on MMT nanoparticles, which is likely due to the easier assembling of the MMT nanodisks at higher concentration, impeding the ASP molecules into the interlayer space of MMT. According to the results, 2 mg/mL was selected for forming the MMT/ASP nanohybrids.

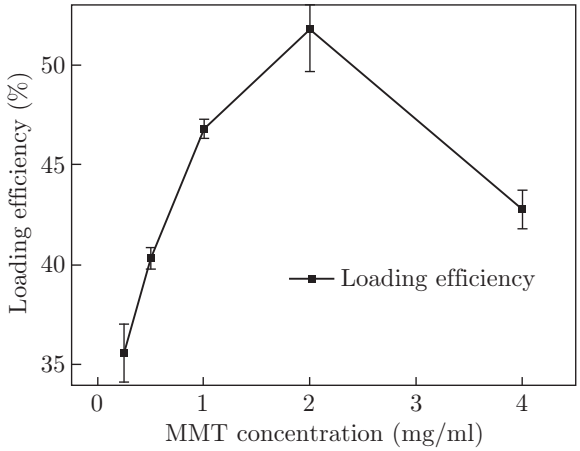


Fig. 1: ASP loading efficiency as a function of MMT concentration

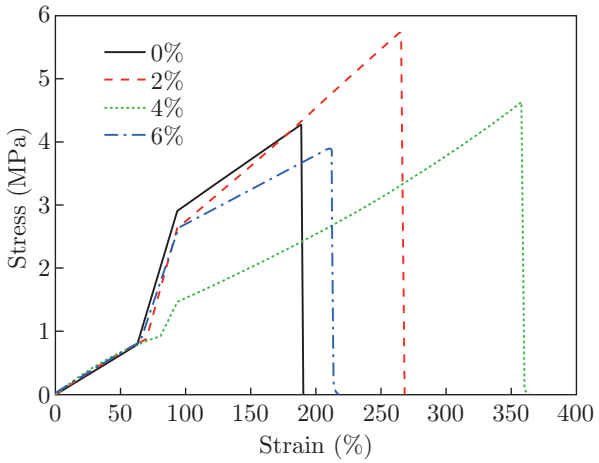


Fig. 2: Typical strain-stress curves of PU nanofibrous mats with 0%, 2%, 4% and 6% of MMT nanoparticles

3.2 Formation of ASP/MMT/PU/Ag Nanofibrous Mats

It is known that mechanical property is important for tissue engineering scaffolds, therefore before producing PU/MMT/ASP/Ag nanofibrous mat, the effect of MMT content on the mechanical

property of PU nanofibrous mats was investigated. Fig. 2 shows the typical stress-strain profiles of PU/MMT nanofibrous mat with different content of MMT nanoparticles. We can see that incorporating MMT into PU nanofibers impacted the mechanical properties of PU nanofibrous mats (see Table 1). For PU nanofibrous mat with MMT, the tensile strength was significantly enhanced, whereas the strain to failure decreased. When the content of MMT was 2%, the tensile strength of PU/MMT nanofibrous mat was 9.55 MPa, 4.24 MPa higher than that of pure PU nanofibrous mats, which might be ascribed to the presence of MMT uniformly dispersed in the PU nanofibers. However, further increasing the content of MMT in PU solution resulted in a gradual decreased tensile strength, although still higher than that of pure PU nanofibrous mats. This might be due to the agglomeration of MMT nanoparticles in the PU solution, which made MMT nanoparticles not disperse in nanofibers uniformly. Therefore, 2% MMT was chose to produce PU/MMT/ASP/Ag nanofibers.

Table 1: Mechanical properties of PU/MMT nanofibrous

Sample	MMT content (%)	tensile stress (MPa)	Stress to failure (%)
PU/MMT nanofibrous mat	0	5.31 ± 0.32	383.50 ± 43.35
	2	9.55 ± 1.96	333.23 ± 36.33
	4	6.73 ± 2.80	232.54 ± 33.84
	6	6.60 ± 2.85	182.68±35.29

The MMT/ASP nanohybrid was incorporated within PU nanofibers (2% ASP relative to PU) via electrospining to form PU/MMT/ASP nanofibers and then Ag nanoparticles was loaded onto fabricated nanofibers to produce PU/MMT/ASP/Ag nanofibers. Meanwhile, PU, PU/ASP (2% ASP relative to PU), PU/MMT and PU/MMT/ASP (2% ASP relative to PU) were prepared as controls. The morphology of the formed electrospun nanofibers with different compositions was characterized using SEM (Fig. 3). It is clear that PU/MMT/ASP/Ag nanofibrous mat shows a 3D porous structure and have uniform, smooth surfaces, except for a slight swelling because of the assembling and soaking processes, similar to the pure PU, PU/ASP and PU/MMT nanofibers. The diameters of PU/MMT (580 nm), PU/MMT/ASP (591 nm), PU/ASP (517 nm) and PU/MMT/ASP/Ag (702 nm) nanofibers are smaller than that of pure PU nanofibers (792 nm), which might be due to the increase of solution conductivity caused by the introduction of charged species in the electrospinning solution [14].

The successful loading of MMT/ASP nanohybrids within PU nanofibers was confirmed using FTIR spectroscopy, which was shown in Fig. 4. Comparing the spectrum of PU/MMT/ASP with that of PU nanofibers, significant difference could be observed. The new peaks emerged at 3640 cm^{-1} ascribed to the —Si—O-stretching vibration of MMT was detected. Except that, the peaks at 1690 cm^{-1} and 1610 cm^{-1} due to vibration of CH_2═CH—COOH and benzene skeleton from ASP were obtained, proving the existence of MMT/ASP nanohybrid in PU nanofibers.

3.3 In Vitro Drug Release

The main idea behind this study is that loading ASP onto MMT nanoparticle can endow them controllable drug release properties. Thus, the study of the drug release kinetics is of vital importance to evaluate the developed new drug delivery systems. The ASP release behaviors of PU/MMT/ASP and PU/ASP nanofibrous mats were investigated in PBS at pH = 7.4. It can be

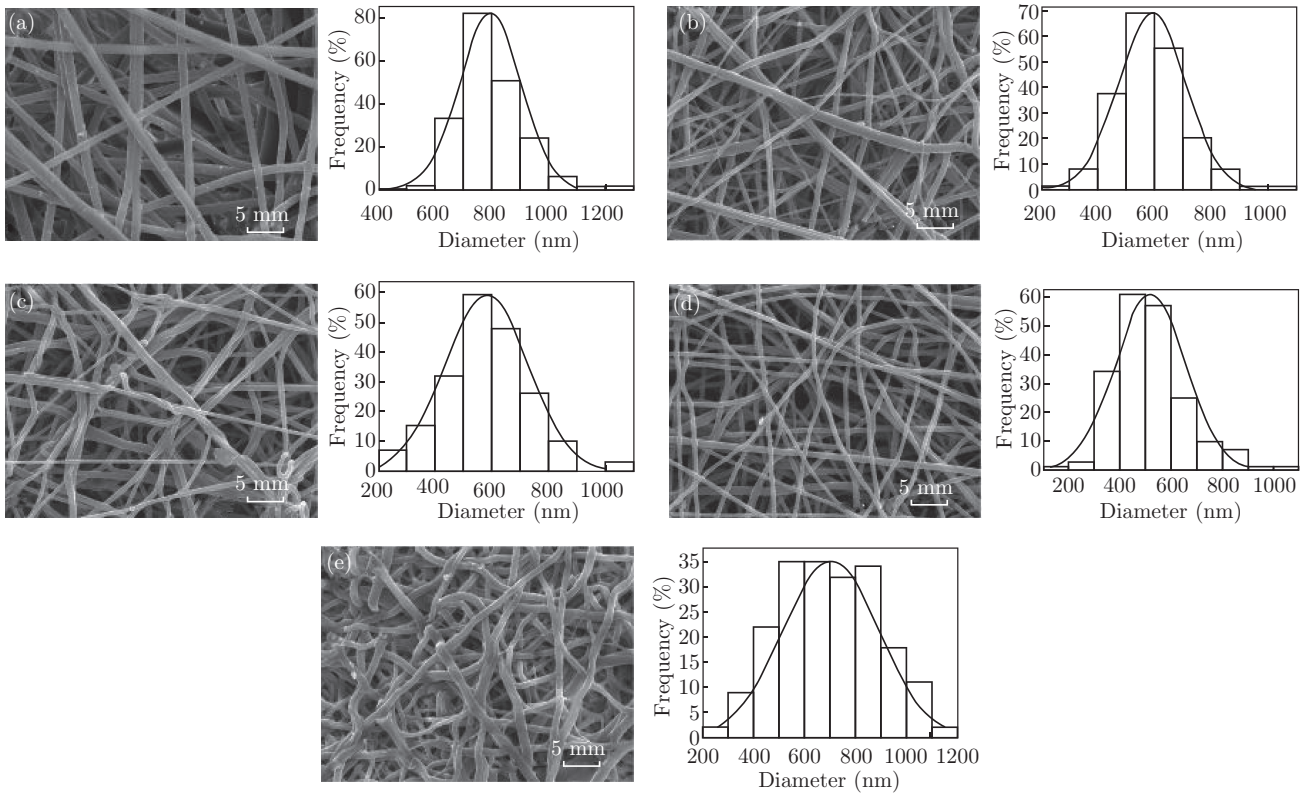


Fig. 3: SEM images and diameter distribution histograms of (a) PU, (b) PU/MMT/ASP, (c) PU/MMT , (d) PU/ASP and (e) PU/MMT/ASP/Ag nanofibers

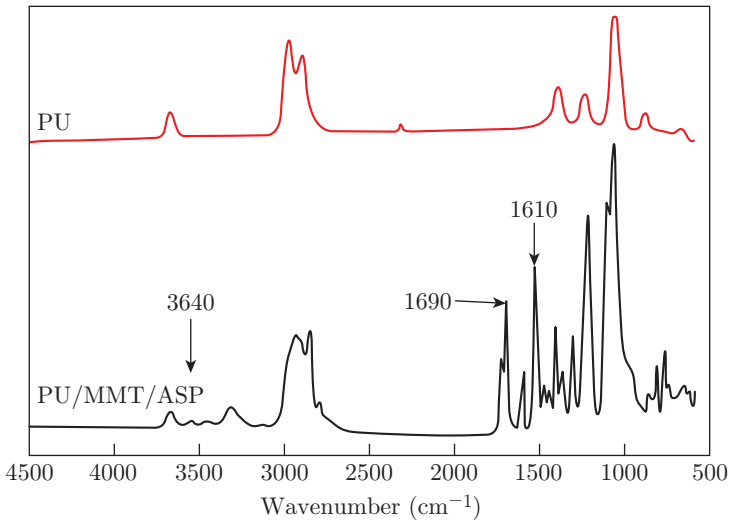


Fig. 4: FTIR spectra of pure PU and PU/MMT/ASP nanofibrous mats

seen from the ASP release profiles of PU/MMT/ASP nanofibrous mats that ASP was released in a sustained manner during 10 days of incubation, the cumulative drug release was 23%. In addition, the initiative drug release was 10.5% of the loaded drug within 5 min, much lower than that from PU/ASP nanofibrous mat (26%), proving that loading ASP onto MMT nanoparticles could significantly mitigate the burst drug release (Fig. 5). This is because that drug should first run out from the reservoir of MMT and then from the polymer matrix, which forms an additional

obstacle for the drug release.

3.4 Cytotoxicity of the ASP/MMT/PU/Ag Nanofibrous Mats

For tissue engineering applications of the PU/MMT/ASP nanofibrous mats, it is important to testify the cytotoxicity of fabricated fibrous scaffolding material. The viabilities of fibroblasts L929 cells seeded onto TCPs, cover slips, and all nanofibrous scaffolds were shown in Fig. 6. Cell proliferations on B, C, D and E were much better than the cells seeded onto A. The incorporation of MMT and ASP did not have severe influence on the cell viability when comparing to the pure PU nanofibers. Although PU/MMT/ASP/Ag nanofiberous mats showed lower cell viability than those of PU and PU/ASP nanofibrous mats ($^{*}p< 0.05$), it is obviously higher than cells cultured on TCPs ($^{**}p< 0.01$). The good cell proliferation onto nanofibrous scaffolds suggests that these PU-based nanofibrous mats have an excellent biocompatibility to promote cell growth. The morphology of L929 cells cultured on different electrospun nanofibrous scaffolds was observed via SEM imaging (Fig. 7). Cells cultured on all nanofibrous scaffolds displayed a fibroblastic phenotype, indicating that the cells can penetrate and migrate within the scaffolds in a manner similar to native ECM. There is no significant difference among PU/MMT/ASP/Ag, PU/MMT/ASP, PU and PU/ASP, reinforcing that PU/MMT/ASP/Ag has a good cytocompatibility after loading ASP, MMT and Ag nanoparticles.

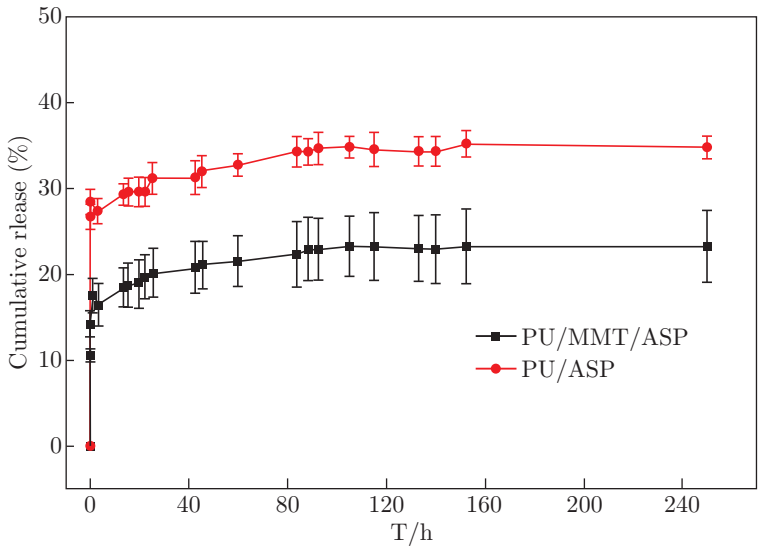


Fig. 5: Drug release profiles of PU/ASP and PU/MMT/ASP nanofibrous mats at pH = 7.4

3.5 Antibacterial Activity Assay

Antibacterial activity of the formed electrospun nanofibers with different compositions were examined using S. Aureus. As shown in Fig. 8, ASP/MMT/PU/Ag composite nanofibrous mat showed a visible inhibition zone (Fig. 8(d), white circle). However, PU and PU/MMT/ASP nanofibrous mats have no antibacterial properties against S. aureus, indicating that the antibacterial activity of developed ASP/MMT/PU/Ag composite nanofibrous mat is solely related to Ag nanoparticles. This may be due to Ag nanoparticles interact with the thiol group of the respiratory enzyme present in the bacterial cell wall.

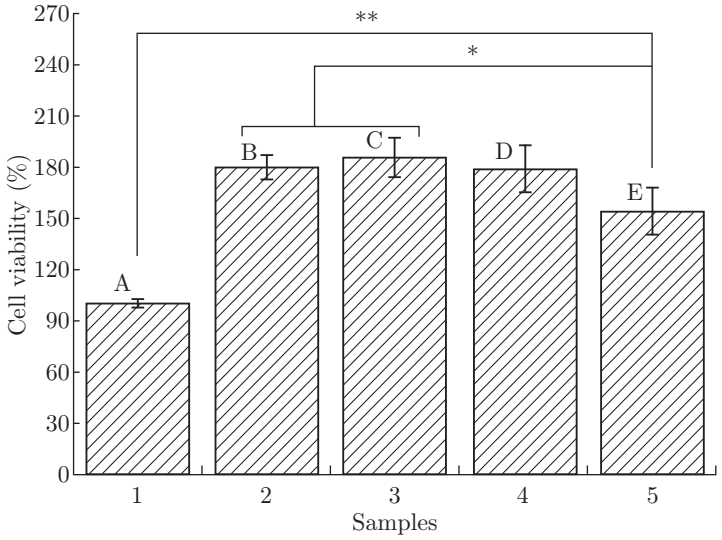


Fig. 6: Rezasurin assay of L929 cells seeded on (A) TCPs, (B) PU, (C) PU/ASP, (D) PU/MMT/ASP and (E) ASP/MMT/PU/Ag nanofibers for 24 h (*p < 0.05, **p < 0.01)

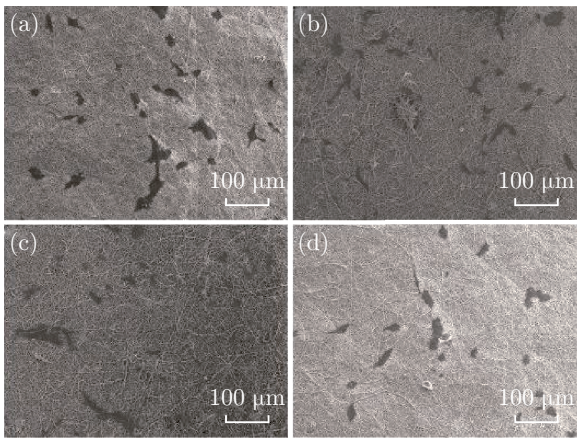


Fig. 7: SEM images of L929 cells cultured onto (a) PU, (b) PU/ASP, (c) PU/MMT/ASP and (d) ASP/MMT/PU/Ag composite nanofibrous mats

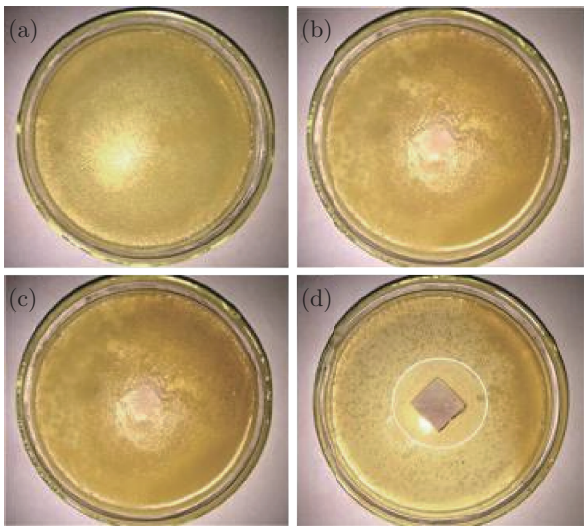


Fig. 8: Photograph of antibacterial activity of (a) control, (b) PU, (c) PU/MMT/ASP and (d) PU/MMT/ASP/Ag nanofibrous mats incubated for 24 h

4 Conclusion

In summary, we have developed a facile approach to load antipyretic analgesic drugs ASP and antibacterial agent Ag nanoparticles into PU/MMT composite nanofibers for tissue engineering application. The MMT/ASP nanohybrids with the ASP loading efficiency of 51.7% were successfully incorporated into PU electrospun nanofibers. The incorporation of 2% MMT nanoparticles not only improved the mechanical properties of the PU nanofibrous mats, but also weakened the initial burst release characteristics of conventional electrospun polymer fiber-based drug delivery system. The PU/MMT/ASP/Ag nanofibrous mats showed excellent antibacterial property biocompatibility, suggesting that the developed composite system is promising in tissue engineering.

Acknowledgement

This research was financially supported by the National Natural Science Foundation of China (51403165) and the Education Commission of Hubei Province (D20161602).

References

[1] Ramalingam K, Castro R, Pires P, et al. Gene delivery using dendrimer/pDNA complexes immobilized in electrospun fibers using the Layer-by-Layer technique. RSC Adv 2016; 6: 97116-97128

[2] Marx V. Tissue engineering: Organs from the lab. Nature 2015; 522: 373-377

[3] Ramakrishna S, Fujihara K, Teo WE, et al. Electrospun nanofibers: solving global issues. Mater Today 2006; 9: 40-50

[4] Barnes CP, Sell SA, Boland ED, et al. Nanofiber technology: designing the next generation of tissue engineering scaffolds. Adv Drug Deliv Rev 2007; 59: 1413-1433

[5] Lannutti J, Reneker D, Ma T, et al. Electrospinning for tissue engineering scaffolds. Mater. Sci. Eng., C 2007; 27: 504-509

[6] Sill TJ, von Recum HA. Electrospinning: applications in drug delivery and tissue engineering. Biomaterials 2008; 29: 989-2006

[7] Jayasinghe SN. Cell electrospinning: a novel tool for functionalising fibres, scaffolds and membranes with living cells and other advanced materials for regenerative biology and medicine. Analyst 2013; 138: 2215-2223

[8] Tan L, Hu J, Zhao H. Design of bilayered nanofibrous mats for wound dressing using an electrospinning technique. Mater Lett 2015; 156: 46-49

[9] Stone SA, Gosavi P, Athauda TJ, et al. In situ citric acid crosslinking of alginate/polyvinyl alcohol electrospun nanofibers. Mater Lett 2013; 112: 32-35

[10] Kim SE, Heo DN, Lee JB, et al. Electrospun gelatin/polyurethane blended nanofibers for wound healing. Biomed Mater 2009; 4: 044106

[11] Tong HW, Zhang X, Wang M. A new nanofiber fabrication technique based on coaxial electrospinning. Mater Lett 2012; 66: 257-260

[12] Detta N, Errico C, Dinucci D, et al. Novel electrospun polyurethane/gelatin composite meshes for vascular grafts. J Mater Sci: Mater Med 2010; 21: 1761-1769

[13] Kenawy ER, Bowlin GL, Mansfield K, et al. Release of tetracycline hydrochloride from electrospun poly (ethylene-co-vinylacetate), poly (lactic acid), and a blend. J Control Release 2002; 81: 57-64

[14] Wang S, Zheng F, Huang Y, et al. Encapsulation of amoxicillin within laponite-doped poly (lactic-co-glycolic acid) nanofibers: preparation, characterization, and antibacterial activity. ACS Appl Mater Inter 2012; 4: 6393-6401

[15] Verreck G, Chun I, Rosenblatt J, et al. Incorporation of drugs in an amorphous state into electrospun nanofibers composed of a water-insoluble, nonbiodegradable polymer. J Control Release 2003; 92: 349-360

[16] Wenhua L, Guangjie Z. Design of wood/montmorillonite (MMT) intercalation nanocomposite. Forest Sci Practice 2004; 6: 54-62

[17] Kevadiya BD, Bajaj HC. The layered silicate, montmorillonite (MMT) as a drug delivery carrier. Key Eng. Mater 2013; 571: 111-132

[18] Fudala A, Palinko I, Kiricsi I. Preparation and characterization of hybrid organic- inorganic composite materials using the amphoteric property of amino acids: amino acid intercalated layered double hydroxide and montmorillonite. Inorg Chem 1999; 38: 4653-4658

[19] Kimmer D, Slobodian P, Petras D, et al. Polyurethane/multiwalled carbon nanotube nanowebs prepared by an electrospinning process. J Appl Polym Sci 2009; 111: 2711-2714

[20] Shah MSAS, Nag M, Kalagara T, et al. Silver on PEG-PU-TiO2 polymer nanocomposite films: an excellent system for antibacterial applications. CHEM MATER 2008; 20: 2455-2460

[21] Dowling DP, Donnelly K, McConnell ML, et al. Deposition of anti-bacterial silver coatings on polymeric substrates. THIN SOLID FILMS 2001; 398: 602-606

[22] Liu H, Zhang Y, Han Y, et al. Characterization and cytotoxicity studies of DPPC: M 2+ novel delivery system for cisplatin thermosensitivity liposome with improving loading efficiency. Colloid Surface B 2015; 131: 12-20

[23] Luo Y, Wang S, Shen M, et al. Carbon nanotube-incorporated multilayered cellulose acetate nanofibers for tissue engineering applications. Carbohyd Polym 2013; 91: 419-427

[24] Djurisic AB, Leung YH, Tam KH, et al. Defect emissions in ZnO nanostructures. Nanotechnology 2007; 18: 095702

[25] Xiao S, Shen M, Guo R, et al. Immobilization of zerovalent iron nanoparticles into electrospun polymer nanofibers: synthesis, characterization, and potential environmental applications. J Phys Chem C 2009; 113: 18062-18068

[26] Bao Y, Ma J, Li N. Synthesis and swelling behaviors of sodium carboxymethyl cellulose-g-poly(AA-co-AM-co-AMPS)/MMT superabsorbent hydrogel. Carbohydr Polym 2011; 84: 76-82

[27] Mansoori Y, Atghia SV, Zamanloo MR, et al. Polymer-clay nanocomposites: free-radical grafting of polyacrylamide onto organophilic montmorillonite. Eur Polym J 2010; 46: 1844-1853

[28] Nair BP, Sindhu M, Nair PD. Polycaprolactone-laponite composite scaffold releasing strontium ranelate for bone tissue engineering applications. Colloid Surface B 2016; 143: 423-430