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Fabrication of polymer/drug-loaded hydroxyapatite particle composite fibers for drug sustained release

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ABSTRACT: The aim of this study is to fabricate polymer/hydroxyapatite (HA) particle composite fibers for drug encapsulation and sustained release. Firstly, drug-loaded hydroxyapatite particles are synthesized in one step, then by electrospinning of the blends of drug-loaded hydroxyapatite particles and polymer solution the drug-loaded polymer/hydroxyapatite particle composite fibers are successfully prepared. Effect of loading ratio of drug-loaded hydroxyapatite particles in the fibers and pH value of the release medium on the drug release kinetics are both investigated, and the results demonstrate that, as compared with the polymer/drug electrospun fibers, the drug in the polymer/drug-loaded hydroxyapatite particle composite fibers shows a sustained release manner, and the drug release rate can be regulated by both the loading ratio of drug-loaded hydroxyapatite particles in the composite fibers and pH value of the buffer solution. The results indicate that the developed drug-loaded polymer/hydroxyapatite particle composite fibers show great potential in bone regeneration and other related biomedical fields. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 42871.

KEYWORDS: biomaterials; biomedical applications; electrospinning

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INTRODUCTION

In recent years, it had been well recognized that the electrospun fiber had become one of the most promising localized drug carrier,[1,2] because various therapeutic agents, such as drugs, genes, and growth factors could be all incorporated into the fibers for controlled release,[3–5] and the drug release profiles could be finely controlled by modulation of the fiber's morphology, porosity, and composition. Moreover, some previous works had demonstrated that the drug bioavailability could be greatly improved after loading in the electrospun fibers.[6] Therefore, electrospun fiber was a promising drug carrier and showed great potential in a range of biomedical applications, including wound healing, prevention of postoperative adhesion, cancer treatment, and tissue engineering.[7–9]

Current approaches for fabrication of drug-loaded electrospun fibers were blending electrospinning, emulsion electrospinning, and coaxial electrospinning, respectively.[10–12] Among these methods, blending electrospinning was the most simple and straight forward method; however, serve burst release was always occurred, which limited its further applications.[13] For the coaxial electrospinning and emulsion electrospinning, both of them could achieve sustained release of drugs, but coaxial electrospinning always needed complex setups, and the kind and the feeding rate of inner fluid and outer fluid should be finely controlled, otherwise, homogenous drug-loaded core-shell structured fibers could not be obtained.[14] And for emulsion electrospinning, the emulsifier, which was used to avoid phase separation, might introduce some biocompatibility issues and influence the drug release profiles. Recently, a novel method for achieving drug sustained release from the drug-loaded electrospun fibers was presented by fabrication of polymer/drug-loaded particle composite fibers. In this kind of drug delivery system, the drug was firstly encapsulated into the particles, followed by incorporated into the polymer matrix by electrospinning, therefore, the particles and the polymer matrix both hindered the drug diffusion, which resulted in a sustainable drug release. Until now, various kinds of particles had been incorporated into the fibers to achieve a sustained drug release, including poly(methyl methacrylate) (PMMA) nanoparticles, halloysite nanotubes, chitosan nanoparticles, mesoporous silica nanoparticles, and poly(lactic-co-glycolic acid) (PLGA) nanoparticles.[15–19] However, for some specific applications, such as bone regeneration and bone tissue engineering, both the drug sustained release and osteoconductivity were highly desired.[20,21] Unfortunately, the above mentioned particles could only load and slow the drug release, and the osteoconductivity of these particles was poor. In order to develop a novel electrospun fiber with both the sustained drug release property and osteoconductivity, a commonly used inorganic material, hydroxyapatite particle, was introduced into the polymer fibers to achieve this goal. Hydroxyapatite (HA), the major inorganic constituent of human bones and teeth, had been

extensively used for hard tissue applications due to its excellent biocompatibility and good osteoconductive capability.[22] Additionally, it had also been proven that HA was an ideal drug carrier for delivering drug, proteins and genes.[23–25]

In this study, ibuprofen (IBU), a highly used anti-inflammatory drug, was chosen as the model drug. Firstly, the ibuprofen-loaded hydroxyapatite (IBU-HA) particles were synthesized in one step, then, the polymer/IBU-HA particle composite fibers containing different amount of IBU-HA particles were fabricated by the electrospinning technique, and effect of IBU-HA particle loading ratio and pH value of the release medium on the drug release profiles of polymer/IBU-HA particle composite fibers were further carried out.

EXPERIMENTAL

Calcium chloride dehydrate ($CaCl_2$ $2H_2O$), disodium hydrogen phosphate dodecahydrate (Na_2HPO_4 $12H_2O$), sodium hydroxide (NaOH), absolute ethanol, and tetrahydrofuran (THF) were all obtained from Tianjin Hedong Hongyan Chemical Reagent Factory. *N,N*-Dimethylformamide (DMF) was purchased from Tianjin Tianli Chemical Reagents Co. PLGA (M_W=150,000, lactic acid : glycolic acid=90 : 10) was purchased from Jinan Daigang Bio-technology Co. IBU was purchased from Tokyo Kasei Kogyo Co. All the reagents were used as received without any further purification.

Fabrication of Ibuprofen-Loaded Hydroxyapatite (IBU-HA) Particles

About 1.0315 g IBU and 0.368 g $CaCl_2$ $2H_2O$ were firstly dissolved into 65 mL anhydrous ethanol. Then, 7 mL deionized water, 3 mL 1*M* NaOH, and 25 mL 0.02*M* Na_2HPO_4 $12H_2O$ solution were added into the above solution in sequence, after stirring for 5 min, the as-synthesized white suspension was separated by centrifugation at 8000 rpm for 5 min. The precipitate was washed twice with deionized water and finally dried in oven at 60°C for 12 h.

To measure the amount of IBU loading in the HA particles, firstly, 0.1 g IBU-HA composite particles were added into 50 mL of 2*M* hydrochloric acid solution. After ultrasonic dispersion for 30 min, the suspension was then stirred for another 24 h until it became transparent. Furthermore, the absorbance of the solution at 264 nm was measured and the concentration of the IBU solution could be calculated based on the standard curve of free IBU solution. The drug loading capacity (DLC) was calculated from the following equation:

$$DLC=A_1/A_2$$

where A_1 and A_2 represented the weight of IBU loaded in HA and the weight of HA, respectively.

Fabrication of PLGA/IBU-HA Particle Electrospun Composite Fibers by Electrospinning

The PLGA/IBU-HA particle composite fibers with different loading ratios of IBU-HA particles (10%, 15%, and 20%) were prepared by using the conventional electrospinning technique. In a typical synthesis procedure, 0.1105 g IBU-HA particles were added in the mixture solvent of 3.0 mL DMF and 1.0 mL THF, then 1.1006 g PLGA was added into the above suspension, after vigorous stirring for 2 h, the spinning suspension was then transferred into a syringe for the further electrospinning. The electrospinning parameters were shown as follows. The feeding rate of the suspension in the syringe was controlled at 1.0 mL/h, the supplied voltage was 13.0 kV and the distance between the tip of the needle and the conductive plate was fixed at 20 cm. Similar procedures were applied to fabricate the PLGA/IBU electrospun fibers. All the electrospinning process was conducted at about 20°C and a relative humidity of 60%.

In Vitro Drug Release Study

The drug-loaded electrospun fibrous mats were firstly cut with a surgical knife into a size of 3 × 3 cm^2, and the weight was accurately measured by an analytical balance with 0.1 mg resolution, and then four pieces of the samples were immersed into the release medium of phosphate buffer solution (PBS, pH 7.4) under the temperature of 37°C. The IBU release profiles from IBU-HA particles and PLGA/IBU electrospun fibers were also carried out as controls. The release medium was removed for analysis at given time intervals and immediately replaced with an equal volume of fresh release medium to keep the total volume constant. The absorbance value of the extracted solution at 264 nm was monitored by using UV–vis spectrometry. Based on the standard curve of IBU solution, the released percentage of IBU could be calculated and plotted as curves versus time according to the following equation:

$$Y=X_1/X_2$$

where Y, X_1, and X_2 represented the released percentage of IBU, the weight of IBU released, and the weight of total IBU loaded, respectively.

Similar procedures were employed to investigate the IBU release kinetics from PLGA/IBU-HA particle electrospun composite fibers in the release medium with different pH values (pH 4.0, 6.0, and 7.4).

Characterization

The morphology of the IBU-HA particles and drug-loaded electrospun fibers was observed under a scanning electron microscopy (SEM, Hitachi, S4800). The average diameters of the four kinds of electrospun fibers were measured by the software of Nanomeasure 1.2. The X-ray powder diffraction (XRD) patterns were recorded by using an X-ray diffractometer (Rigaku D/max 2550 V) with high-intensity Cu Kα radiation. Water contact angles of the samples were measured by using a Data Physics OCA 20 Tensiometer at 25°C. The volume of an individual water droplet was fixed at 5 μL, and the water contact angle was determined by measuring the same sample at five different positions. FTIR spectra were recorded with a Bruker Tensor 27 FTIR spectrometer in the range of 4000–400 cm^{-1} using KBr pellets.

RESULTS AND DISCUSSION

Characterization of IBU-HA Particles

Figure 1(a) showed the XRD pattern of IBU-HA particles, from the result it could be found that the diffraction peaks corresponded well to the standard for HA (JCPDS No. 09-0432), however, no obvious diffraction peaks of IBU could be observed. The small amount of IBU in the IBU-HA composite particles might lead to

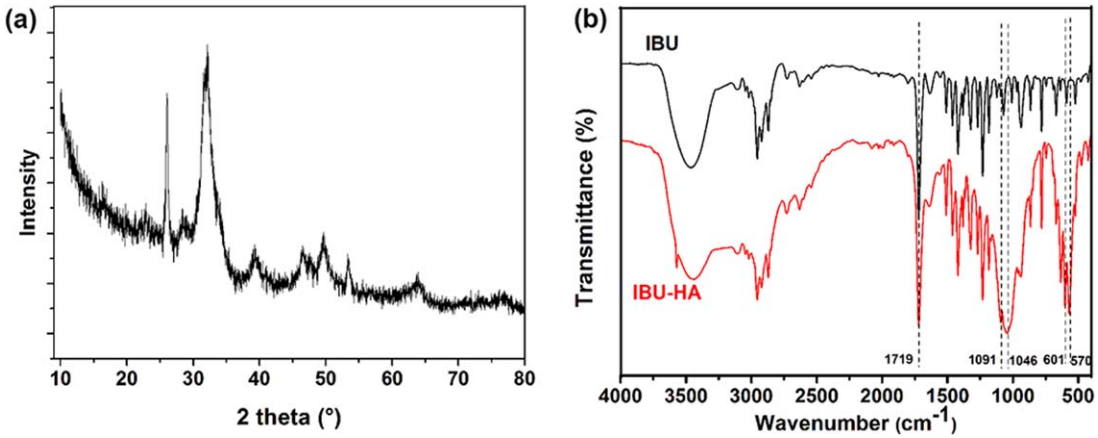

Figure 1. XRD pattern of IBU-HA particles (a) and FTIR spectra of IBU and IBU-HA particles (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

relative low intensity peaks, which might be overlapped by the strong peaks of HA in the XRD pattern. In order to confirm the existence of IBU in the IBU-HA composite particles, the FTIR spectra of both IBU and IBU-HA composite particles were recorded and shown in Figure 1(b). In the FTIR spectrum of IBU, the strong carbonyl absorption band at 1719 cm^{-1} could be clearly observed. By contrast, in the FTIR spectrum of the IBU-HA composite particles, the characteristic peaks at 1719 cm^{-1} implied the presence of IBU in the IBU-HA composite particles. In addition, the peaks at 1091, 1046, 601, and 570 cm^{-1} were attributed to the phosphate groups, which indicated the existence of HA in the IBU-HA composite particles. Overall, the above results demonstrated that IBU was successfully incorporated into the HA particles. The loading capacity of HA for IBU was also measured by dissolving the IBU-HA composite particles with excess dilute hydrochloric acid solution, following by measuring and calculating the concentration of IBU in the solution based on the standard curve of free IBU solution, and the result indicated that the loading capacity of HA particles for IBU was 0.087g/g. Figure 2 showed the morphology of the obtained IBU-HA particles. From the SEM images, it could be clearly observed the particles were long spindle in shape with the length of 400–800 nm and the width about 300 nm.

Morphology and Structure of the Drug-Loaded Electrospun Fibers

The drug-loaded electrospun fibers were fabricated by the electrospinning technique and the morphology of the fibers was shown in Figure 3. From Figure 3(a) it could be found that the PLGA/IBU electrospun fibers had homogenous structure, and the surface of the fibers was smooth and no drug crystal was found on the surface. However, surface structure of the electrospun fibers varied apparently after adding the IBU-HA particles [Figure 3(b–d)]. As seen from the PLGA/IBU-HA particle composite fibers, some bulgings were observed on the fibers due to the larger size of IBU-HA particles than the electrospun composite fibers. In addition, from Figure 4 it could be found that the average diameters of the four kinds of electrospun fibers were gradually increased. Previous studies had demonstrated that the viscosity, electrical conductivity, and surface tension of the spinning solution all had great influence on the diameter of the resultant electrospun fibers.[26–28] In our study, the viscosity, electrical conductivity, and surface tension of the spinning solution might be changed after adding the different amount of IBU-HA composite particles, which would resulted in an increased fiber diameter.

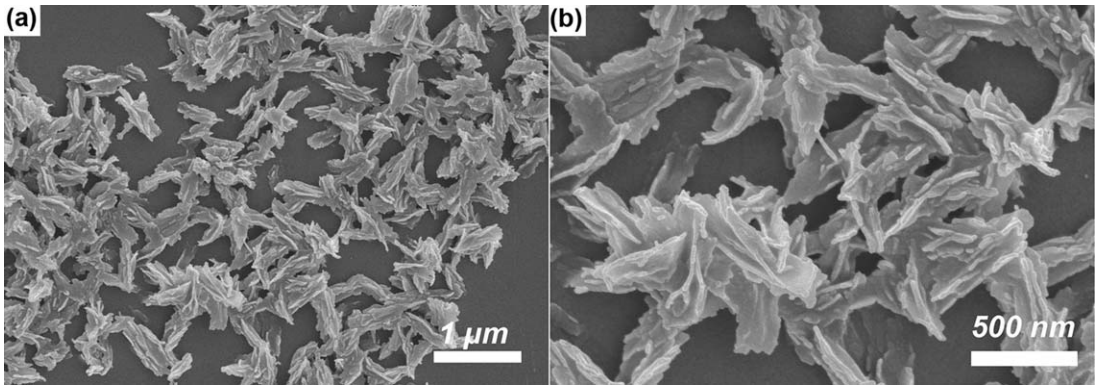

Figure 2. SEM images of IBU-HA particles (a: low magnification; b: high magnification).

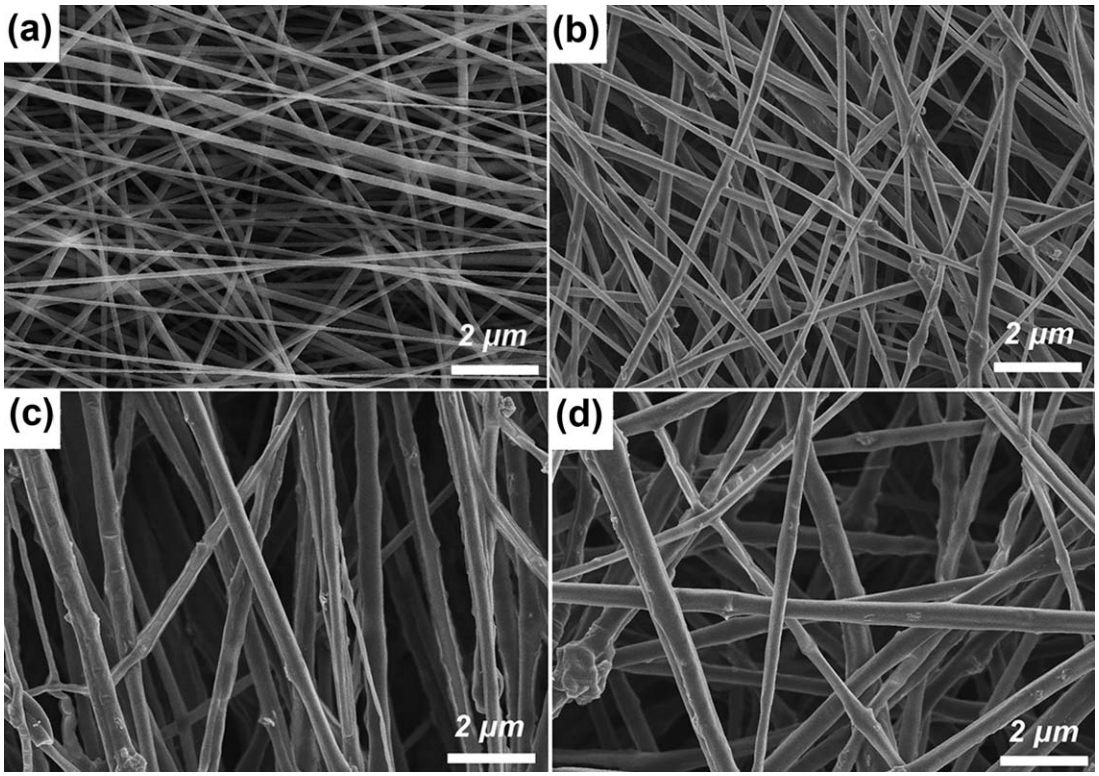

Figure 3. SEM images of PLGA/IBU electrospun fibers (a), PLGA/10% IBU-HA particle composite fibers (b), PLGA/15% IBU-HA particle composite fibers (c), and PLGA/20% IBU-HA particle composite fibers (d).

Drug Delivery Profiles

The release characteristic of IBU from PLGA/IBU-HA particle composite fibers was carried out, and the release behaviors of the IBU-HA particles and PLGA/IBU electrospun fibers were also conducted as controls. As seen in Figure 5, it could be observed that the drug IBU in the IBU-HA particles and PLGA/IBU electrospun fibers both had a serve burst release, followed by a sustained release profile. After incubation in PBS for 24 h, 60.5% of IBU was released from IBU-HA particles and a total of 85.4% was released after 324 h, which was very similar with the release profile of IBU from IBU-loaded hierarchical HA microspheres, and the drug release kinetic showed a biphase drug release property.[29] Similarly, for PLGA/IBU electrospun fibers almost 93.5% of the drug was released over a period of 324 h. By contrast, PLGA/IBU-HA particle composite fibers with different loading ratios were all released sustainably. After releasing for 324 h, 22.5%, 31.5% and 41.3% of IBU

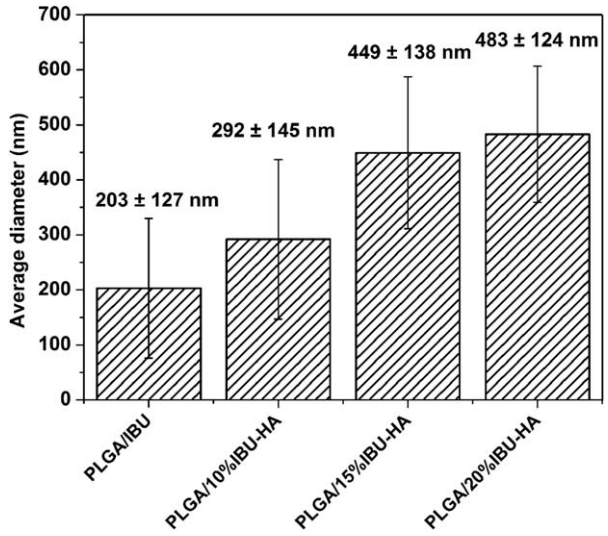

Figure 4. The average diameters of PLGA/IBU electrospun fibers, PLGA/10% IBU-HA particle composite fibers, PLGA/15% IBU-HA particle composite fibers and PLGA/20% IBU-HA particle composite fibers.

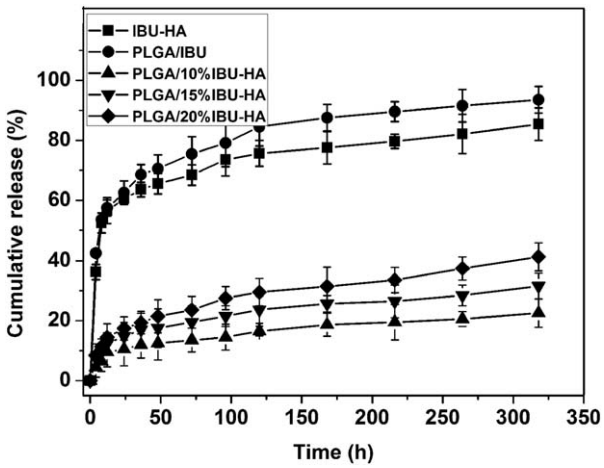

Figure 5. The cumulative release of IBU from IBU-HA particles, PLGA/IBU composite fibers, PLGA/10% IBU-HA particle composite fibers, PLGA/15% IBU-HA particle composite fibers, and PLGA/20% IBU-HA particle composite fibers.

were released, respectively. From these data, it could be also found that the release rate of IBU increased with the increasing of IBU-HA particles loading in the composite fibers, that is, the release profile of IBU could be finely controlled by the loading ratio of the IBU-HA particles in the electrospun fibers.

In this study, the PLGA/IBU electrospun fibers were fabricated by the conventional blending electrospinning, and the drug IBU was easily enriched on or near the surface of the fiber, therefore, IBU in the PLGA/IBU electrospun fibers showed a serve burst release. However, after incorporating the IBU-HA particles into the electrospun fibers, two barriers would hinder the drug release, one was the HA particles and the other was the polymer fibers. That is, once the PLGA/IBU-HA particle composite fibers were incubated into the PBS, the drug IBU would firstly desorb from the HA particles, followed by diffusing from the polymer fibers, thus, the drug IBU showed a sustainable release profile from PLGA/IBU-HA particle electrospun fibers. As mentioned above, the drug release rate increased with the increasing amount of IBU-HA particles in the electrospun fibers. As it is known that HA has excellent hydrophilic characterization, therefore, after being incorporated into the electrospun fibers, the hydrophilicity of electrospun composite fibers could be improved (Figure 6), which resulted in a faster release. In addition, as the amount of IBU-HA particles loading in the composite fibers increased, more IBU-HA particles would locate near the surface of the fiber, which would also accelerate the drug release.

In vitro release experiments were performed in buffer solution at pH values of 4.0, 6.0, and 7.4, respectively. As shown in Figure 7, at pH 7.4 the percentage of IBU released was reached to about 10% within the first 24 h, and eventually reached to 22.5% after 324 h. By contrast, the pH 6.0 environment induced a relatively faster release, after incubation in buffer solution for 324 h, 29.6% of the IBU was released. At pH 4.0, IBU released 36.3% of the total drug over a period of 324 h. These results demonstrated that the release rate of IBU from PLGA/IBU-HA particle electrospun composite fibers was increased when the pH value of the release medium was decreased. The rapid IBU release might attribute that HA degraded faster in the acid medium than in the physiological environment.[30] With the decrease of pH from 7.4 to 4.0, more HA would dissolve, and the drug encapsulated in the IBU-HA particles were easily diffused out, followed by releasing from the polymer fibers.

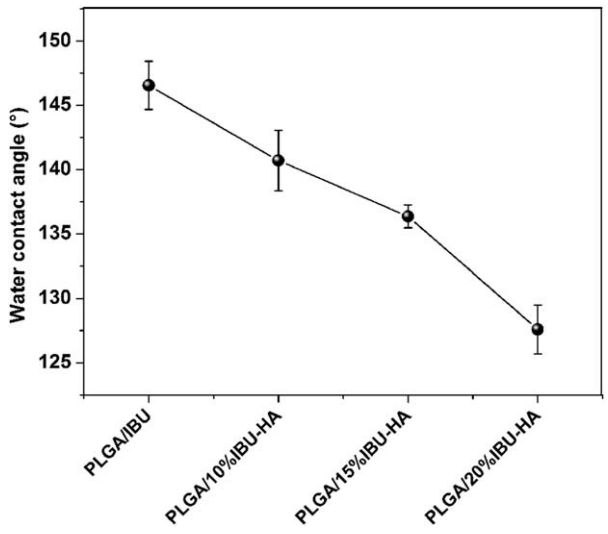

Figure 6. Water contact angle of PLGA/IBU electrospun fibers, PLGA/10% IBU-HA particle composite fibers, PLGA/15% IBU-HA particle composite fibers, and PLGA/20% IBU-HA particle composite fibers.

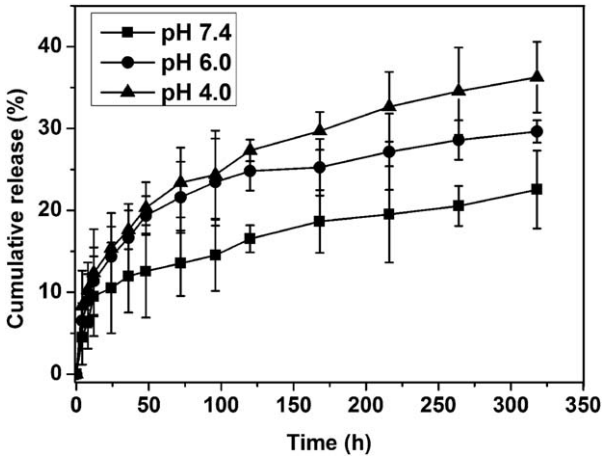

Figure 7. Release profiles of IBU from PLGA/10% IBU-HA particle composite fibers in the release medium with the pH value of 4.0, 6.0, and 7.4, respectively.

Recently, due to the osteoconductive capability of HA, several research groups had already successfully fabricated polymer and HA composite nanofibers by electrospinning for bone repairing.[31,32] However, it was insufficient to just embed HA particles in the polymer nanofibers for bone healing, because the inflammation was frequently occurred in the lesion location and this kind of composite nanofibers did not have the anti-inflammation effect. In order to solve these problems mentioned above, in this study, both the anti-inflammatory drug IBU and HA were incorporated into the polymer nanofibers. This multifunctional drug-loaded electrospun composite fiber could not only accelerate bone repairing but also prevent and diminish inflammation. Moreover, the results also demonstrated that the release of drug could be controlled by changing the pH of the release medium, which was of great physiological significance, because the pH value of inflammatory tissues was lower than that of the normal tissues.

CONCLUSIONS

The PLGA/IBU-HA particle composite fibers with different loading ratio of IBU-HA particles were successfully fabricated and the results indicated that the composite fibers showed relatively homogenous structure and IBU-HA particles were well encapsulated within the polymer fibers. The drug release studies indicated the drug IBU in the PLGA/IBU-HA particle composite fibers showed a sustained release behavior. Moreover, the loading ratio of IBU-HA particles and the pH value of the release medium greatly influenced the release profiles, and the drug release rate increased with the increasing of loading ratio of IBU-HA particles in the fibers and decreased with the increasing of the pH value of the release medium. The developed PLGA/

HA particle composite fibers with controllable drug delivery property and excellent bioactivity might have potential in bone regeneration and bone tissue engineering.

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