In Vitro Degradation and Drug-Release Behavior of Electrospun, Fibrous Webs of Poly(lactic-*co*-glycolic acid)

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ABSTRACT: Ultrafine fibrous webs of poly(lactide-*co*-glycolic acid) (PLGA) containing the bactericidal antibiotic drug rifampin were prepared by electrospinning, and their properties were investigated for wound-dressing applications. Because PLGA is a biodegradable and biocompatible polymer, it is one of the best materials for the preparation of wound-dressing substrates. Through this investigation of PLGA/rifampin electrospun webs, we found that the *in vitro* degradation reached approximately 60% in 10 days, and the drug release from the webs showed a fast and constant profile suitable for wound-dressing applications. Also, we observed that both the web-degradation rate and the drug-release rate increased as the drug concentration in the PLGA/rifampin electrospun webs and the content level of glycolide units in the PLGA polymer matrix increased. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 209–214, 2012

Key words: biodegradable; drug delivery systems; fibers

INTRODUCTION

Over the past few years, there has been increasing interest in ultrafine polymer fibers for biomedical applications. In particular, drug-impregnated, biodegradable, ultrafine fibers are very effective for topical drug administration and wound healing because the ultrafine fibrous webs have unique properties, such as high surface-to-volume ratios, small pore sizes, and high porosity. It has been proven that the drugs impregnated into ultrafine fibrous webs are released at various controlled rates and with various profiles based on the morphology of the fibers and the drug concentration in the fibrous webs. Moreover, the use of biodegradable, biocompatible materials such as poly(lactic acid), poly(glycolic acid), and their copolymer poly(lactide-*co*-glycolic acid) (PLGA) reduces the risk of unwanted toxicities and adverse effects.[1] Also, biodegradable polymers offer more disposal options and are more environmentally friendly to manufacture in comparison with traditional petroleum-based plastics. PLGA is one of the most popular biodegradable polymers approved by the U.S. Food and Drug Administration and has been widely investigated for its applicability in drug-delivery applications[2–6] because of its suitable biodegradability, biocompatibility, and mechanical properties.[7] On the other hand, there are several techniques used for the production of polymeric ultrafine fibers, such as electrospinning, phase separation,[8,9] template synthesis,[10] and self-assembly.[7,11,12] In particular, electrospinning has evinced more interest in recent years because of its versatility and potential for applications in diverse fields. In the electrospinning process, a polymer solution or melt is subjected to strong electric fields, and then the liquid-phase polymer is ejected from a nozzle. The diameter of the ejected fibers is significantly reduced as they travel toward a collector. Hence, electrospun, fine fibers offer various advantages such as high surface-area-to-volume ratios and tunable porosity, and the ultrafine fiber composition can be manipulated to obtain desired properties and functions.

Therefore, in this study, we prepared PLGA ultrafine fibrous webs impregnated with the antibiotic drug rifampin via the electrospinning process (PLGA/rifampin electrospun webs), and we investigated their biodegradable properties and drug-release profiles for use in wound-dressing applications. In particular, we expected this kind of material to be very effective for wound healing because unlike oral antibiotics, locally applied antibiotics can target and kill harmful bacteria before they enter the body to cause further infection, sepsis, or death.[13]

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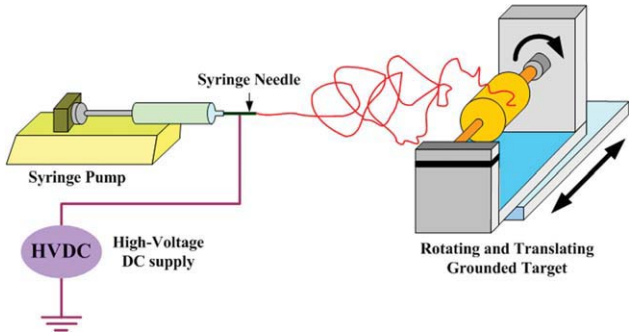

Figure 1 System setup for the electrospinning process. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

EXPERIMENTAL

Materials

PLGA (50 : 50) with a weight-average molecular weight of 71,000 and PLGA (65 : 35) with a weight-average molecular weight of 114,000 were purchased from Medisorb (Alkermes, Wilmington, OH). Rifampin (purity $\geq$ 97%; high-performance liquid chromatography) in a powder form was obtained from Sigma–Aldrich Korea (Yongin, Korea). Tetrahydrofuran, dimethylformamide, and other chemicals were purchased from Ducksan Pure Chemical Co. (Gyeonggi-Do, Korea). All reagents were used as received without any further purification.

Preparation

For the preparation of ultrafine PLGA fibers containing rifampin, PLGA and rifampin were dissolved in a 2 : 8 dimethylformamide/tetrahydrofuran mixed solvent in designated amounts, and then the solutions were electrospun into a nonwoven web state. The electrospinning setup used in this study consisted of a hypodermic syringe (20-gauge needle), a copper electrode, an aluminum collecting drum, and a high-voltage supply (Chungpa EMI, Seoul, Korea), as shown in Figure 1. A syringe pump connected to the hypodermic syringe controlled the flow rate. The polymer solutions were electrospun at positive voltages ranging from 13 to 22 kV, the tip-to-collector distance was 18.5 cm, and the solution flow rate was approximately 4 mL/h. After the electrospinning, the nonwoven samples were stabilized under atmospheric conditions (25 $\pm$ 5°C and 65% relative humidity) for 24 h, and then they were vacuum-dried for 7 days for complete removal of the residual solvents. Moreover, we used two PLGA polymers, PLGA (50 : 50) and PLGA (65 : 35), which were classified by their ratios of lactic acid to glycolic acid.

Characterization

The surface morphologies of the PLGA/rifampin electrospun webs were examined with a field emission scanning electron microscope (JSM 6330F, JEOL, Japan). Using the scanning electron microscopy (SEM) images, we measured the fiber diameters and obtained the average fiber diameter. The release of rifampin from the electrospun webs was monitored with an Evolution 600 ultraviolet–visible spectrophotometer (Thermo Scientific, United States) at the wavelength of 473 nm according to Zeng et al.'s study.[14] The fiber samples were each incubated in a 100-mL phosphate buffer solution (pH 7.4) containing 10 μg/mL of proteinase K (40 U/mg) at 37°C under shaking (120 rpm) for various periods of time. For the control test, no proteinase K was added. At a certain time, 50 mL of the buffer solution was taken out, and an equal amount of fresh buffer solution was added to the incubation solution. We read the absorption values (at 473 nm) of the buffer solutions taken out at intervals, and we converted the absorption values into rifampin concentrations according to a calibration curve of rifampin in the same buffer. Then, the accumulative amount of the released rifampin was calculated as a function of the incubation time.[14] To determine the biodegradability of the PLGA/rifampin electrospun webs, we measured the weight of the electrospun webs at certain time intervals during the drug-release assessment. The fiber relaxation was calculated with the following formula:

$$\text{Fiber relaxation } (\%) = \frac{D_a - D_b}{D_b} \times 100 \qquad (1)$$

where D_b and D_a are the average fiber diameters of the electrospun webs before and after immersion in the buffer solution for 2 days (μm), respectively.

RESULTS AND DISCUSSION

Degradability of the PLGA/rifampin electrospun webs

Effect of the drug concentration

We studied the PLGA/rifampin electrospun webs as a function of the rifampin concentration, and we investigated the degradability of the electrospun webs in a phosphate buffer solution containing protease K for 10 days. Figure 2 shows the time profile of the weight loss of the PLGA (50 : 50)/rifampin electrospun webs as the rifampin concentration increased in the webs (0, 4, 8, 12, and 16 wt %). The electrospun webs were degraded with increasing time, and the extent of the degradation increased as the rifampin concentration in the webs increased. The addition of rifampin decreased the fiber

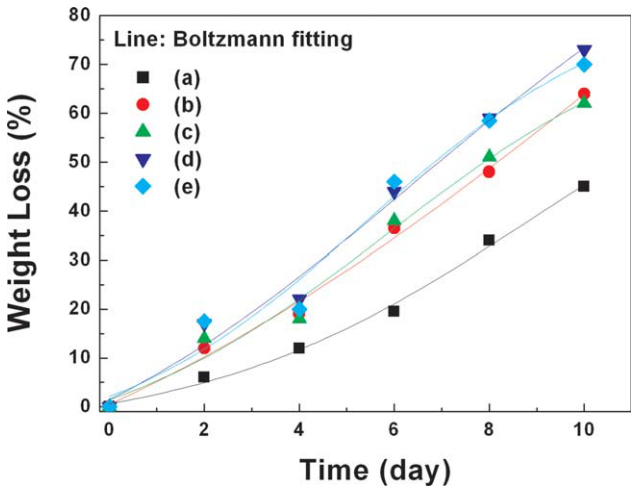

Figure 2 *In vitro* degradation behavior of 100% PLGA (50 : 50)/rifampin electrospun webs as a function of the rifampin concentration: (a) 0, (b) 4, (c) 8, (d) 12, and (e) 18%. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

diameter in the PLGA/rifampin electrospun webs, as shown in Figure 3(a) for the PLGA (50 : 50) electrospun web (average diameter = 2.50 μm) and in Figure 4(a) for the PLGA (50 : 50)/rifampin electrospun web (average diameter = 1.93 μm), because rifampin enhanced the polarizability of the polymer dope solution during the electrospinning.[14,15] Therefore, the effective surface area of the PLGA/rifampin electrospun webs also increased with increasing rifampin concentration, and this induced the increase in the degradation rate of the webs.[16]

Figures 3 and 4 show the surface morphologies of the PLGA (50 : 50) electrospun web and PLGA (50 : 50)/4% rifampin electrospun web with increasing time in the buffer solution. From the images, we observed that the electrospun webs obviously degraded with increasing time, and the degradation rate of the PLGA (50 : 50)/4% rifampin electrospun fibers was much faster than that of the PLGA (50 : 50) electrospun fibers. This result was consistent with the previous weight-loss percentage results (Fig. 2). On the other hand, after the initial immersion of the webs in an aqueous buffer solution [Figs. 3(b) and 4(b)], the electrospun fibers in both webs were significantly swollen, and this indicated that polymer relaxation happened in the fibers. The relaxation ratio of the PLGA (50 : 50)/4% rifampin electrospun fibers (70%) was greater than that of the PLGA (50 : 50) electrospun fibers (54%). Apparently, a decrease in the surface tension of the PLGA (50:50)/rifampin electrospun webs occurred upon the addition of the hydrophilic drug rifampin, which led to improved wetting of the PLGA (50 : 50) fibers in an aqueous solution. Therefore, the PLGA (50 : 50)/rifampin electrospun fibers probably formed a

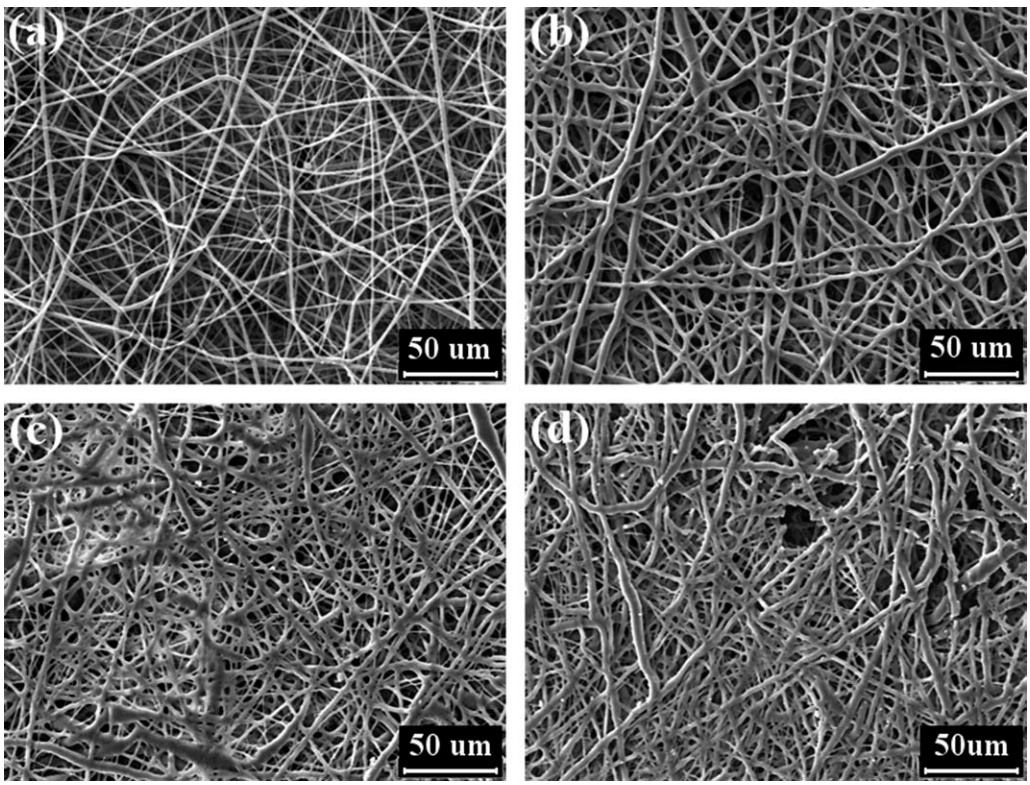

Figure 3 SEM images of PLGA (50 : 50) electrospun webs as a function of the incubation time: (a) 0, (b) 2, (c) 4, and (d) 6 days.

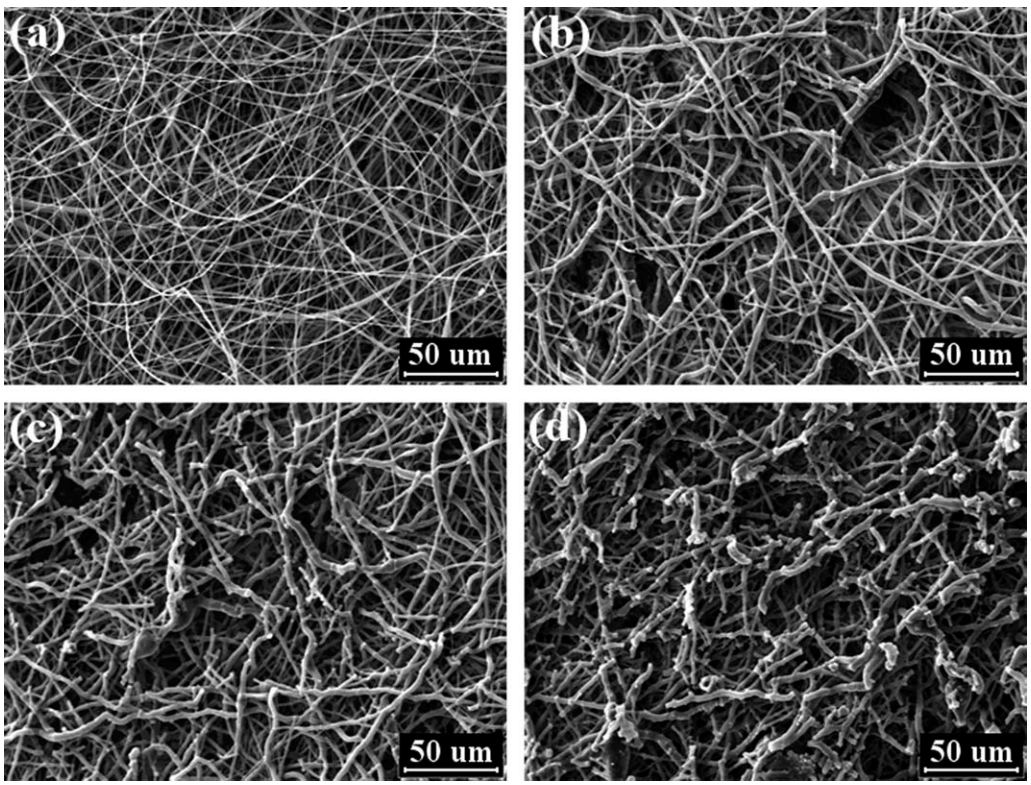

Figure 4 SEM morphologies of PLGA (50 : 50)/4% rifampin electrospun webs as a function of the immersion time: (a) 0, (b) 2, (c) 4, and (d) 6 days.

damping layer on the surface, which facilitated relaxation of the internal stresses and consequently accelerated degradation of the fibers.[17] Also, it was presumed that drug release from the PLGA (50 : 50)/rifampin electrospun webs affected the greater weight loss of the electrospun webs.

Effect of the PLGA composition

We also studied the PLGA/rifampin electrospun webs as a function of the PLGA composition via the blending ratios of PLGA (50 : 50) to PLGA (65 : 35) in the polymer dope solutions during the electrospinning process, and we investigated the degradability of the electrospun webs, as shown in Figure 5. As the PLGA (50 : 50) content increased in the polymer dope solution, the degradation rate increased because the higher the number of glycolide units was, the less time was required for degradation.[18] Therefore, we thought that the degradation rate and drug-release profile of the PLGA/rifampin electrospun webs could be controlled by adjustments of the composition of the PLGA species in the polymer dope solution during the electrospinning process. On the other hand, Figure 6 shows exterior images of 75% PLGA (50 : 50)–25% PLGA (65 : 35)/12% rifampin electrospun webs as the immersion time increased in the buffer solution. The electrospun web significantly shrank after its initial immersion in the buffer solution [Fig. 6(b)], and this phenomenon seems to have a thread of connection to the polymer relaxation shown in Figures 3(b) and 4(b).

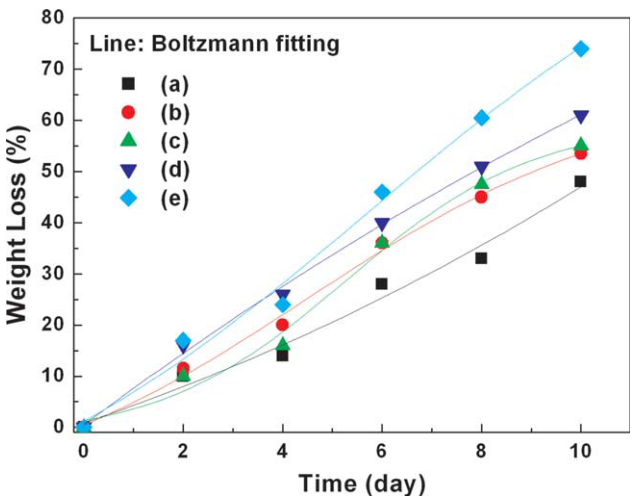

Figure 5 *In vitro* degradation behavior of PLGA/4% rifampin electrospun webs as a function of the PLGA composition: (a) 0% PLGA (50 : 50) and 100% PLGA (65 : 35), (b) 25% PLGA (50 : 50) and 75% PLGA (65 : 35), (c) 50% PLGA (50 : 50) and 50% PLGA (65 : 35), (d) 75% PLGA (50 : 50) and 25% PLGA (65 : 35), and (e) 100% PLGA (50 : 50) and 0% PLGA (65 : 35). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

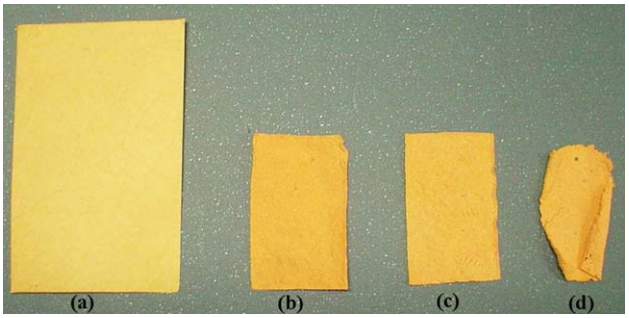

Figure 6 Images of 75% PLGA (50 : 50)–25% PLGA (65 : 35)/12% rifampin electrospun webs as a function of the immersion time: (a) 0, (b) 2, (c) 4, and (d) 6 days. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Drug-release behavior of the PLGA/rifampin electrospun webs

The drug-release assessment was carried out as follows: PLGA/rifampin electrospun webs were incubated in a phosphate buffer solution containing 10 μg/mL proteinase K at 37°C, and the absorbance of rifampin at 473 nm as a function of time was monitored. Figure 7 shows the drug-release profiles of the 100% PLGA (50 : 50)/8% rifampin electrospun web, the 100% PLGA (50 : 50)/12% rifampin electrospun web, and the 100% PLGA (50 : 50)/16% rifampin electrospun web for 10 days. An initial burst release of the drug was further observed as the rifampin concentration increased in the webs. The initial rapid release was attributed to release from a superficial area of electrospun fibers and could involve both dissolution and diffusion, and the second phase could be attributed to the diffusion of the drug from the inside of the electrospun fibers by

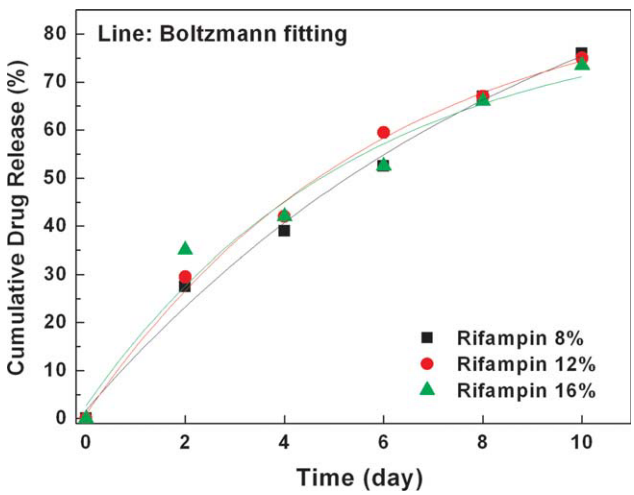

Figure 7 Drug-release profiles from 100% PLGA (50 : 50)/rifampin electrospun webs as a function of the drug concentration. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

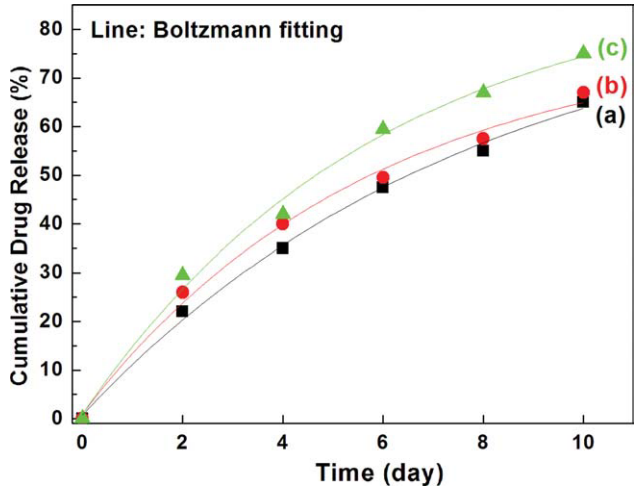

Figure 8 Drug-release profiles from PLGA/12% rifampin electrospun webs as a function of the PLGA composition. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

dissolution of the surface.[19,20] Therefore, it was thought that the higher drug concentration in the polymer dope solution induced the arrangement of more drug on the surface of the PLGA electrospun fibers. This is in agreement with the results of our previous study.[21] Figure 8 shows the drug-release behaviors of the 100% PLGA (65 : 35)/12% rifampin electrospun web, the 50% PLGA (50 : 50)–50% PLGA (65 : 35)/12% rifampin electrospun web, and the 100% PLGA (50 : 50)/12% rifampin electrospun web for 10 days. The drug-release rate increased as the PLGA (50 : 50) content increased in the polymer matrix. Therefore, it was proved that the degradation rate and drug-release behavior of the PLGA/rifampin electrospun webs were decisively controlled by adjustments of the composition of the PLGA species in the polymer dope solution during the electrospinning process.

In addition, to investigate the mechanism of rifampin release from PLGA/rifampin electrospun webs, we performed a release study of the initial data of the 100% PLGA (65 : 35)/12% rifampin electrospun web, the 50% PLGA (50 : 50)–50% PLGA (65 : 35)/12% rifampin electrospun web, and the 100% PLGA (50 : 50)/12% rifampin electrospun web with the Hixson–Crowell model. In general, drug release from a polymer matrix containing water-soluble drugs can be described by Higuchi's model.[22–24] Figure 9 shows plots of rifampin release based on the Hixson–Crowell model and the fitting patterns obtained by the regression analysis with the following equation:

$$Q_0^{1/3} - Q_t^{1/3} = K_s t \tag{2}$$

where Q_0 is the initial amount of the drug in the pharmaceutical dosage form, Q_t is the remaining

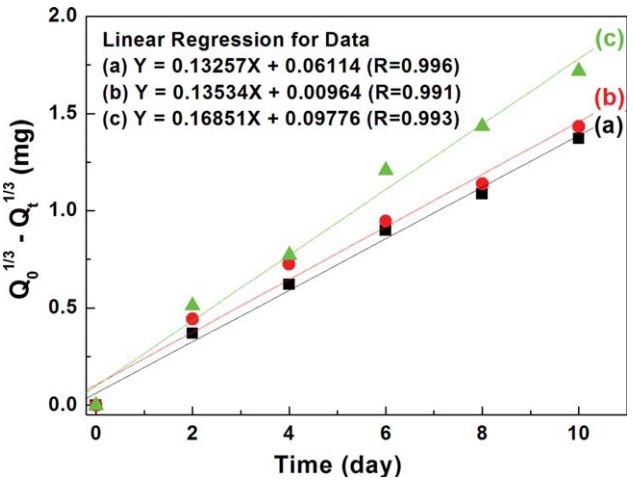

Figure 9 Cube-root plot of the released drug based on the Hixson–Crowell equation: (a) 100% PLGA (65 : 35), (b) 50% PLGA (50 : 50) and 50%PLGA (65 : 35), and (c) 100% PLGA (50 : 50). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

amount of the drug in the pharmaceutical dosage form at time t, and K_s is a constant incorporating the surface–volume relation.

This result suggests that the release of rifampin from a PLGA electrospun web is more controlled by the disintegration of the PLGA electrospun web than the drug diffusion in the PLGA electrospun web.

CONCLUSIONS

Ultrafine fibrous webs of PLGA containing rifampin were successfully prepared by electrospinning. We investigated the degradability and drug-release behavior of the PLGA/rifampin electrospun webs for wound-dressing applications. We found that the *in vitro* degradation of the PLGA/rifampin electrospun webs was superior and that the drug release from the PLGA/rifampin electrospun webs showed a fast and constant profile. Also, we observed that the degradation and the drug-release rate were accelerated by increases in the drug concentration in the PLGA/rifampin electrospun webs and in the number of glycolide units in the PLGA polymer matrix, respectively. Therefore, we found that the characteristics of the PLGA/rifampin electrospun webs in terms of degradation and drug-release behavior could be controlled by the manipulation of the preparation to comply with the applicable requirements of the webs.

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