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Ultraviolet-Crosslinked Hydrogel Sustained-Release Hydrophobic Antibiotics with Long-Term Antibacterial Activity and Limited Cytotoxicity

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ABSTRACT: To prolong erythromycin (EM) release and prevent the side effects of EM, a Pluronic F-127 diacrylate macromer (PF127) was synthesized and then self-assembled into micelles with their hydrophobic cores loaded with EM. The EM-loaded micelles were mixed with a photoinitiator to form the EM/PF127 hydrogels rapidly under a low-intensity UV light. Afterward, the hydrogel properties, antibacterial performance, and cytotoxicity of this novel hybrid hydrogel were investigated. The results show that the EM/PF127 hydrogel had a rapid gelation time. The sustained release of EM reduced its side effects. With controlled antibacterial activity, the use of EM would be safer and more efficient. What is more, the EM/PF127 hydrogel showed a slight cytotoxicity, and this suggests great potential application as antibacterial hydrogels in the prevention of postoperative infection. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40438.

KEYWORDS: composites; crosslinking; drug-delivery systems; gels; photopolymerization

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INTRODUCTION

Erythromycin (EM; CAS: 80214-83-1, Figure 1), a hydrophobic lactone antibiotic, has attracted a great deal of attention for the treatment of infectious diseases in the last decades.[1] Several transdermal administration forms, such as liposomes, emulsions, micelles, and microspheres, have been explored to deliver EM through the skin.[2–5] However, the delivery to and maintenance of prolonged EM release at the site of periprosthetic inflammation presents a considerable challenge.

In recent years, hydrogels have attracted increasing interest in the delivery hydrophobic drugs because of their good biocompatibility, controlled and prolonged drug release, and so on.[6–12] For example, Ma and Zhang[13] conjugated a hydrophobic drug (indomethacin) with poly(ethylene glycol) to form a prodrug and then mixed it with α-cyclodextrin to form an indomethacin *in situ* loaded supramolecular hydrogel. Zhong et al.[14] first encapsulated hydrophobic glycyrrhetinic acid in poly(D,L-lactic) microspheres, then dispersed them in an alginate hydrogel. Zhang et al.[15] prepared a hyperbranched polyester hydrogel that could load hydrophobic drug molecules within the hyperbranched polyester cavities to realize a sustained drug release.[15] Ma et al.[16] encapsulated camptothecin in the micelles formed by Pluronic F-127 in an aqueous solution and then obtained a hydrophobic-drug-loaded hydrogel after mixed with α-cyclodextrin. Li et al.[17] prepared a stearic acid-modified methylcellulose hydrogels for hydrophobic drug loading and prolonged release.

However, most reported hydrogels have usually needed rigorous or longtime reaction conditions. In this study, the EM-loaded hydrogel was prepared rapidly with Pluronic F-127 as the matrix. Previous work has confirmed that diacrylate macromers could homopolymerize or copolymerize to form hydrogels under a low-intensity UV light.[18,19] In this article, Pluronic F-127 diacrylate macromer (PF127) was synthesized and then used to self-assemble into micelles with their hydrophobic cores loaded with EM. The EM-loaded micelles were then mixed with the photoinitiator to form hydrophobic-drug-loaded hydrogels rapidly under a low-intensity UV light. The properties of the

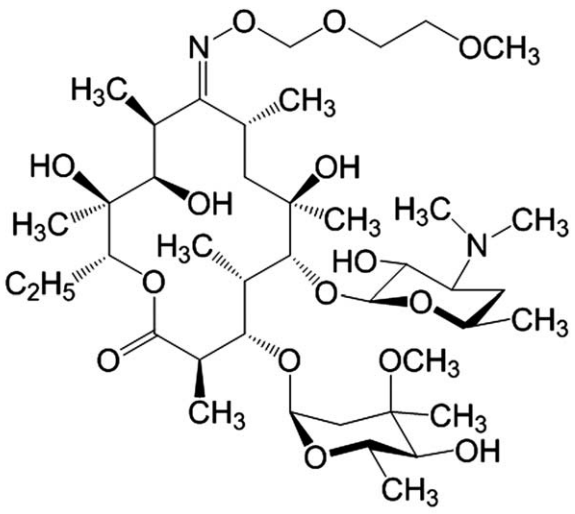

Figure 1. Chemical structure of EM.

hydrogels, such as their strength, swelling, and *in vitro* drug release, were also studied. Then, the antibacterial performance of this novel hydrogel was investigated for both Gram-positive and Gram-negative bacteria, and the sustained-release capability, long-term antibacterial effects, and lower cytotoxicity of this novel hydrogel were demonstrated.

The novelty of this study was that the use of the PF127 hydrogel as the EM carrier prolonged EM release and made the use of EM safer. The specific benefits of this novel EM/PF127 hybrid included (1) dose limitation to prevent eukaryotic toxicity; (2) dose control to achieve the desired antibacterial effects; (3) controllability of the product lifetime, that is, the end of antibacterial activity before the dissolution and diffusion of the hydrogel; and (4) sustained-release delivery to enhance its therapeutic potential while reducing its side effects.

EXPERIMENTAL

Materials

Pluronic F-127 (ethyleneoxide $(EO)_{100}$–propyleneoxide $(PO)_{65}$–ethyleneoxide $(EO)_{100}$) was purchased from Sigma and used after dried *in vacuo* at 60°C for 24 h. Acryloyl chloride (Schuchardt, Germany) and triethylamine (Tianjin Damao Chemical Reagents Co., China) were distilled just before use. 1-Vinyl-2-pyrrolidone (Merck Schuchardt, Germany) and 2,2-dimethoxy-2-phenylacetophenone (Acros) were used as received. Dichloromethane and acetone (Tianjing Damao Chemical Reagents Co., China) were purified by distillation after dehydration with $CaCl_2$. EM were provided by Guangdong Medical College and were used directly. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538 were supplied by the Guangdong Institute of Microbiology (Guangzhou, China). Luria–Bertani (LB) broth and nutrient agar culture medium were supplied by Huankai Microorganism Co., Ltd. (Guangzhou, China). The thiazolyl blue tetrazolium bromide (MTT) substance was purchased from Sigma-Aldrich (Shanghai, China). Mice skin fibroblast (L929) cells were supplied by the General Hospital of Guangzhou Military Command. All other chemicals were analytical grade and were used as received without further purification. All of the aqueous solutions were prepared with ultrapure water (>18 MΩ) from a Milli-Q Plus system (Millipore).

Synthesis of PF127

PF127 was synthesized through reference to Ma et al.'s[19] work. Briefly, 12.6 g of Pluronic F-127 was dissolved in 100 mL of mixed solvent of dry CH_2Cl_2 and acetone (50/50 v/v) in a 250-mL, round-bottomed flask and cooled to 0°C in an ice bath. Then, triethylamine and acryloyl chloride were added dropwise to the solution over 30 min under continuous magnetic stirring. The mixture was stirred at 0°C for 12 h and at room temperature for another 12 h. The byproduct triethylamine hydrochloride was filtered off, and the filtrate was precipitated in an excess of anhydrous ethyl ether. After it was dried at 40°C *in vacuo* for 24 h, PF127 was obtained with a yield of 93%.

^{1}H-NMR ($CDCl_3$): δ = 5.77–6.48 (m, 3H, OCOC*H*CH_2), 3.65–4.24 (m, 800H, OCH_2CH_2O), 3.40 [s, 65H, $OCH_2CH(CH_3)O$], 3.56 [s, 130H, $OCH_2CH(CH_3)O$], 1.14 [s, 195H, $OCH_2CH(CH_3)O$].

Preparation of the EM-Loaded PF127 Micelles

For EM loading, 0.30 g of PF127 was dissolved in 3 mL of distilled water, and then, 5 mL of the EM acetone solution (0.5 mg/mL) was added dropwise in ice-bath conditions. The mixture was stirred at 37°C for 24 h, and the organic solvent was removed by rotary evaporation. The resulting 3-mL mixture was filtered through a 0.45-μm filter to remove the free EM. The particle size and morphology of the EM-loaded PF127 micelles were characterized by a ζ-potential analyzer instrument (ZetaPALS, Brookhaven) and transmission electron microscopy (TEM; JEM-2010HR).

The loading amount of EM was determined by an High Performance Liquid Chromatography (HPLC) test.[4] The EM-loaded PF127 micelles were collected by centrifugation at 8000 rpm, and the supernatant was withdrawn. The concentration of EM in supernatant was dialyzed [immersed in phosphate-buffered saline (PBS); pH 7.4, with a dialysis bag, molecular weight cutoff = 3000] and detected with an analysis of the active ingredient by a HPLC (Agilent 1260) with an external standard method.

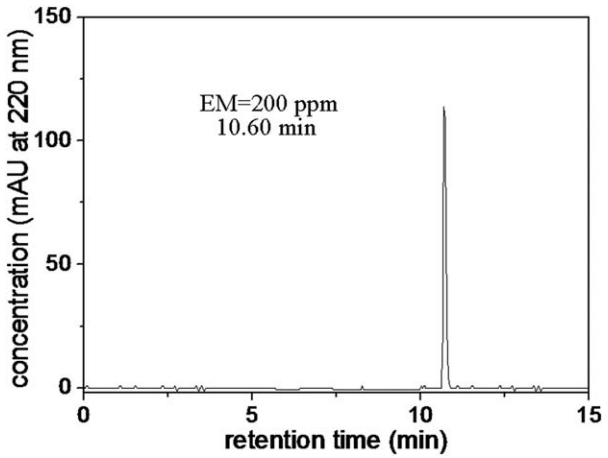

Figure 2. Retention time of EM.

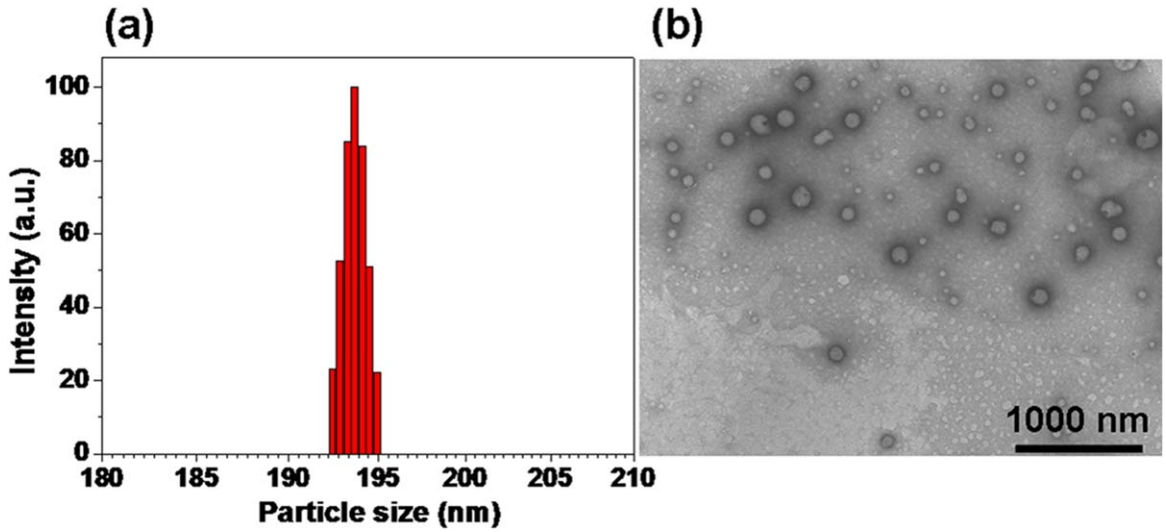

Figure 3. (A) Dynamic Light Scattering (DLS) result and (B) TEM image of EM-loaded PF127 micelles (10% PF127). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The chromatograph conditions of HPLC were as follows: the separation was performed on a AQ-C18 column (size = 4.6 × 250 mm^2, Ultimate), the detection wavelength was set at 220 nm (the reference wavelength was set at 315 nm), the detection temperature was 30°C, and the mobile phase was prepared by the mixture of 700 mL of acetonitrile with 300 mL of a 0.05 mol/L solution of potassium dihydrogenphosphate in water adjusted to pH 7.5 with a 10% sodium hydroxide solution and was degassed with helium (purity = 5.0). The detection limit was 0.4 μg/mL. The retention time of EM was 10.60 min (Figure 2).

Preparation of the UV Crosslinked Hydrogels

For hydrogel formation, 1 mL of 10% EM-loaded PF127 solution (100 mg) was mixed adequately with 50 μL of 2.0% 2,2-dimethoxy-2-phenylacetophenone 1-vinyl-2-pyrrolidone solution in beakers. After that, the beakers were exposed under a UV light from a low-intensity UV lamp (Ex 365 nm, 0.06 mW/cm^2, WFH-203, Shanghai Jingke Industrial Co., Ltd.) for different times (1, 3, and 5 min), and then, the EM-loaded hydrogels were obtained. The resulting EM/PF127 hydrogels were designated as EM/PF127-1 (exposed under UV light for 1 min), EM/PF127-2 (exposed under UV light for 3 min), and EM/PF127-3 (exposed under UV light for 5 min), respectively. Their structure was characterized by scanning electron microscopy (SEM; JSM-6330F scanning electron microscope with an accelerating voltage of 20.0 kV). The swollen hydrogel samples were quickly frozen in liquid nitrogen and then lyophilized. The lyophilized hydrogel samples were fractured carefully, fixed on aluminum stubs, and coated with gold. The dynamic viscoelastic properties of the EM/PF127 hydrogels were studied with a strain-controlled Rheometrics ARES rheometer fitted with a plate–plate tool. The diameter of the plates was 20 mm, and the gap was set to 1.0 mm. All of the measurements were carried out at 37°C. To ensure the rheological measurements within linear viscoelastic regions, a strain sweep test was first carried out. In this case, a strain of 0.05% was determined for linear viscoelastic measurements. To understand the strength properties of the resulting hydrogels, frequency sweep analyses within the range from 0.1 to 50 rad/s were conducted for the hydrogel samples with different UV crosslinked times.

In Vitro Drug Release

The EM/PF127 hydrogel (2 g) was immersed in 50 mL of PBS (0.01 mol/L, pH 7.4) containing 1% Tween-80 at 37°C. At predetermined time points, 500 μL of the medium solution was taken out, and 500 μL of fresh PBS medium was added back to maintain the same total solution volume. The *in vitro* release kinetics of EM from the PF127 micelles was also studied as a control group. A volume of 1 mL of the EM-loaded PF127 micelles was injected into the dialysis bag (molecular weight cutoff = 30,000) and was then immersed into 10 mL of PBS. At predetermined time points, 500 μL of the medium solution was taken out, and 500 μL of fresh PBS was added. The amount of released EM was determined by an HPLC instrument (Agilent 1260) with an external standard method. The percentage of cumulative amounts of released EM was calculated from a

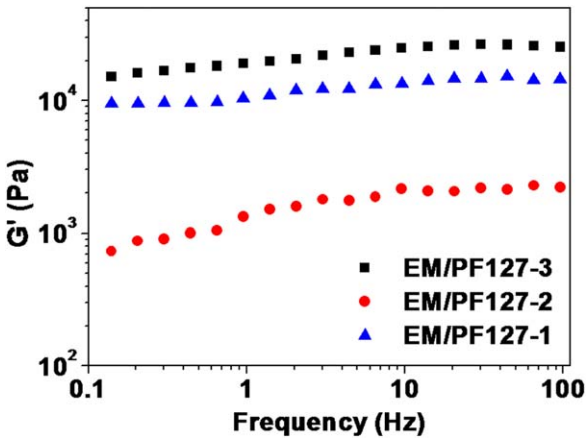

Figure 4. Effect of the crosslinking time on the G' of hydrogel formation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

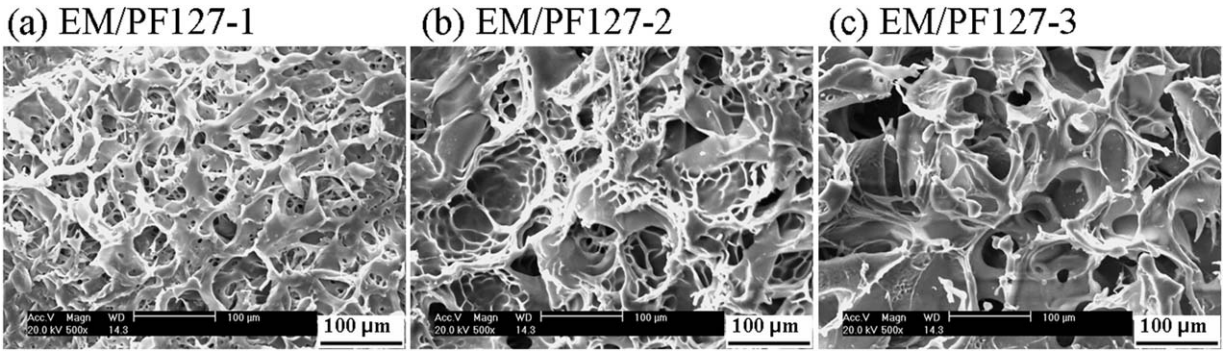

Figure 5. SEM images of the EM/PF127 hydrogels.

standard calibration curve. All of the release studies were carried out in triplicate.

Minimum Inhibitory Concentration (MIC) Tests

The MIC values of the EM, PF127 micelles, and EM/PF127 hydrogel against *E. coli* and *S. aureus* were measured by a twofold dilution method. Briefly, the EM, PF127 micelles, or EM/PF127 hydrogel were suspended into Mueller–Hinton broth medium to form homogeneous suspensions and then twofold diluted into different concentrations. The 1-mL samples of culture medium containing various concentrations of the test samples were each inoculated with 0.1 mL of 10^6 cfu/mL bacterial suspensions and cultured for 24 h at 37°C under shaking. Then, the growth of the bacteria was observed. When no growth of bacteria was observed in the lowest concentration of test sample, the MIC of the sample was defined as this value of dilution. The test for every MIC of EM, PF127 micelles, and EM/PF127 hydrogel was repeated three times.

Inhibitory Zone Tests

E. coli and *S. aureus* were selected as indicators of the experimental bacteria. LB broth was used as a growing medium for both microorganisms *E. coli* and *S. aureus*. All of the culture media and materials were sterilized in an autoclave at 121°C for 30 min before the experiments. An amount of 1.0 g of the EM/PF127 hydrogel was coated on the filter paper (which was 7 mm in diameter). Then, the filter paper was taken out, placed in the middle of the LB agar plates, and incubated at 37 ± 1°C for 24 h. The reaction of the microorganisms to the EM/PF127 hydrogel was determined by the size of the inhibitory zone.

Cytotoxicity Assay

The cytotoxicity of the EM/PF127-2 was tested with the MTT assay on the basis of the cellular uptake of MTT and its subsequent reduction in the mitochondria of living cells; this generated dark blue MTT formazan crystals. L929 cells were seeded on 96-well plates (1.5–2 × 10^4 cells/well) in corresponding media. Then, the cells were treated with PF127, EM, or EM/PF127-2 for 24 h. After that, MTT (5 mg/mL in PBS) was added to each well and incubated for additional 4 h (37°C, 5% CO_2). The cells were then lysed in dimethyl sulfoxide (150 μL/well), and the plates were allowed to stay in the incubator (37°C, 5% CO_2) to dissolve the purple formazan crystals. The color intensity reflecting the cell viability was read at 490 nm with a model-550 enzyme-linked immunosorbent microplate (Bio-Rad), and the morphological changes of the L929 cells were photographed by an IX-70 inverted-phase contrast microscope (Olympus, Japan). All of the experiments were repeated four times, and the Statistical Product and Service Solutions software was used to assess the statistical significance of the differences among the treatment groups.

Statistical Analysis

Statistical analysis was performed with Statistical Product and Service Solutions software statistical software (SPSS 11.0). The differences between the groups were assessed with the analysis of variance test. The results were considered statistically significant when the p value was less than 0.05 or less than 0.01.

RESULTS AND DISCUSSION

EM-Loaded Micelles

PF127 was first modified by bisacryloyl groups, and then used to load EM into its hydrophobic core in aqueous solution. The loading amount was analyzed by HPLC and determined to be 38.6 μg/mL, and the drug encapsulation efficiency was 28.3%. The size and morphology of the EM-loaded PF127 micelles (EM/PF127) was tested by Dynamic Light Scattering (DLS) and TEM analysis (Figure 3). It was found that the size of EM/PF127 was about 193.6 nm, and the micelles showed a spherical morphology.

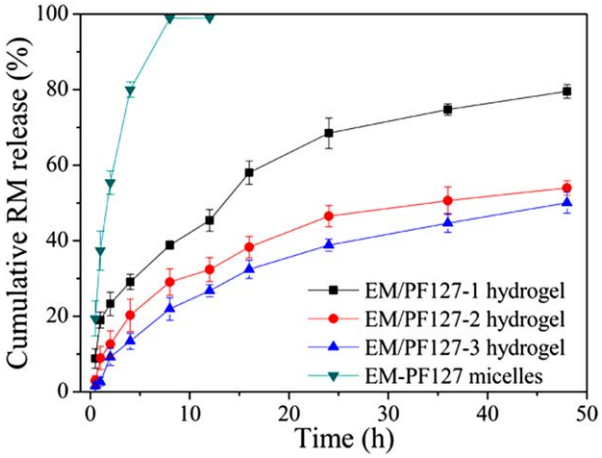

Figure 6. Cumulative EM release profiles of the EM/PF127 micelles and EM/PF127 hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. EM Release Kinetic Parameters

Hydrogel sample	K	n	R^2	Transport mechanism
EM/PF127-1	0.78 ± 0.07	0.38 ± 0.01	0.98	Non-Fickian diffusion
EM/PF127-2	0.69 ± 0.03	0.48 ± 0.01	0.97	Non-Fickian diffusion
EM/PF127-3	0.64 ± 0.04	0.59 ± 0.03	0.99	Non-Fickian diffusion

EM/PF127 Hydrogel Strength

After they were exposed under UV light for a few minutes, the EM/PF127 micelles were mixed with a photoinitiator crosslinked to form the hydrogel samples. To understand the hydrogel formation and hydrogel strength, a dynamic frequency sweep was carried out on the hydrogels formed with different crosslinking time, and the results are shown in Figure 4. As shown, the storage modulus (G') value of the resulting hydrogels increased with increasing crosslinking time; this suggested that the hydrogel strength increased with exposure time under a UV light. At a frequency of 1.0 rad/s, for example, the G' value increased from 1204 to 14,210 Pa when the crosslinking time was increased from 1 to 5 min. This result was attributed to the higher crosslinking points of the hydrogel network with the crosslinking time; this would result in a higher crosslinking density of the hydrogels. Moreover, after 1 min of crosslinking, the hydrogel had already formed, and the hydrogel strength was up to 1000 Pa; this indicated that this micelle rapidly formed a hydrogel.

EM/PF127 Hydrogel Structure

SEM was used to study the structure. As shown in Figure 5, the crosslinking density of the resulting hydrogels increased with increasing crosslinking time. This suggested that the hydrogel crosslinking density increased with exposure time under the UV light. This result coincided with those of the dynamic frequency sweep and was attributed to the higher crosslinking points of the hydrogel network with the crosslinking time; this resulted in a higher crosslinking density of the hydrogels.

In Vitro Drug Release

Figure 6 shows the *in vitro* release profiles for the loaded EM released from the EM/PF127 micelles and EM/PF127 hydrogels in PBS. To represent the controlled and sustained drug release for supramolecular hydrogels, the release profiles of EM from the micelles were studied. The release rate of EM from the micelles was much faster than that from the hydrogels, and the release ratio was over 90% after 8 h. In the EM/PF127 hydrogels, EM could be released in a sustained manner, and no initial burst release was obvious. Depending on the crosslinking time used for the hydrogel formation, various release rates were found for the loaded EM. As shown, the release rate of EM decreased with increasing crosslinking time. This phenomenon was explained by the consideration of the formation of a denser hydrogel network in the case of the longer crosslinking time. This hindered the release of RM from the hydrogels.

To understand the release mechanism of the loaded RM, we fitted the accumulative release data with the following semiempirical equation:[20]

$$\frac{M_t}{M_\infty} = Kt^n \left(for \frac{M_t}{M_\infty} \leq 0.6 \right) \quad (1)$$

where M_t/M_∞ is the fraction of drug released, K is a constant dependent on the system, t is the release period, and n is the diffusion exponent, which is indicative of the release mechanism for matrices of various shapes and swelling or nonswelling systems.

According to the semiempirical eq. (1), an n value of 0.45 indicates Fickian diffusion, where the drug is released by the usual molecular diffusion through the system, and an n value between 0.45 and 0.89 is indicative of anomalous transport, in which there must be some influence of swelling and/or degradation (non-Fickian diffusion). An n value of 0.89 indicates a case II relaxational mechanism, which is associated with the stresses and state transitions that occur in the swelling.

When $\log(M_t/M_\infty)$ versus $\log t$ was plotted, the n, K, and the corresponding determination coefficients (R^2) were obtained, as listed in Table I. The K values were found to decrease with increasing crosslinking time. This phenomenon could be explained by the greater gelation extent and the formation of a denser hydrogel network in the case of a longer crosslinking time, which hindered the EM release and prevented hydrogel erosion. In addition, the n values in all of the hydrogel cases were found to be in the range from 0.38 to 0.59; this showed a non-Fickian diffusion mechanism and indicated that the drug release must have been influenced by swelling and degradation.[21]

MIC Analysis

Table II shows the MIC values of the EM, EM/PF127 micelles, and EM/PF127 hydrogels. EM showed excellent antibacterial activity against *E. coli* and *S. aureus* because both of the MIC values were less than 0.05 mg/mL. The EM/PF127 micelles also showed good antibacterial activity against *E. coli* and *S. aureus*. For the EM/PF127 hydrogels, they showed a relatively high

Table II. MIC Values of the TC, PF127/TC Micelles, and PF127/CD/TC Supramolecular Hydrogels

Sample	MIC (mg/L)	
	E. coli	*S. aureus*
EM	0.5	0.3
EM-PF127 micelles	2.5	1.5
EM/PF127-1 hydrogel	20	15
EM/PF127-2 hydrogel	60	50
EM/PF127-3 hydrogel	80	65

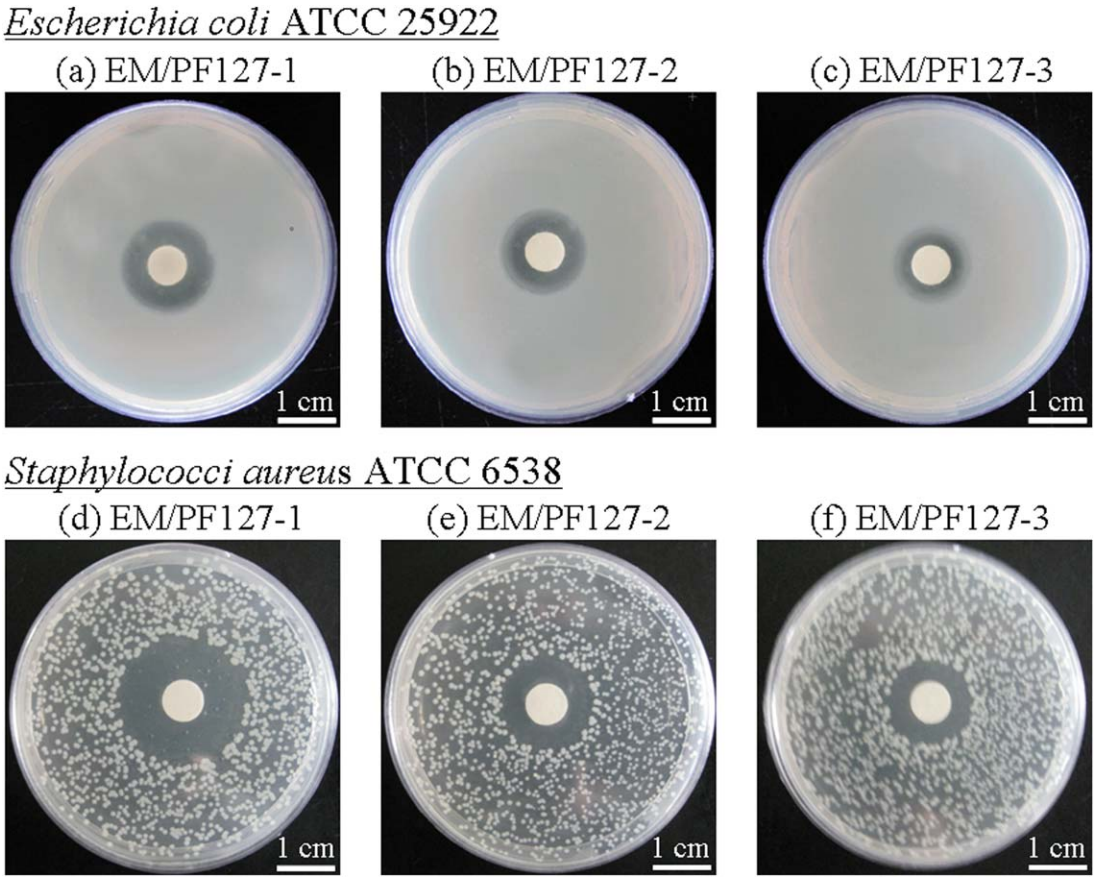

Figure 7. Photographs of the inhibitory zone tests of (a) EM/PF127-1, (b) EM/PF127-2, and (c) EM/PF127-3 against *E. coli* and (d) EM/PF127-1, (e) EM/PF127-2, and (f) EM/PF127-3 against *S. aureus*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

antibacterial activity against *E. coli* and *S. aureus*, and the antibacterial activity was enhanced along with decreasing crosslinking time because a longer crosslinking time hindered the greater release of EM. Because the EM/PF127 hydrogels had a controlled product lifetime, that is, the end of antibacterial activity before their dissolution and diffusion, the EM/PF127 hydrogels more easily achieved desired antibacterial effects compared to the EM/PF127 micelles and EM.

In addition, all of the samples exhibited a lower activity against *E. coli* than against *S. aureus*. The structure of the cytoderm of *E. coli* was more complicated than that of the *S. aureus* because the other layer outside of the peptidoglycan layer is called the outer membrane; this was composed mainly of lipopolysaccharides and phospholipids. The outer membrane took a significant role in protecting bacteria cells against foreign compounds such as EM. Thus, the lower sensitivity of the EM/PF127 hydrogels toward *E. coli* was mainly due to the presence of the outer membrane.[22–24]

Inhibitory Zone Tests

Figure 7 shows the inhibitory zones of the EM/PF127 hydrogels after 24 h of contact with *E. coli* and *S. aureus* when the visible bacteria colonies in the blank sample were about 10^5 to 10^6 cfu/mL. The EM/PF127 hydrogels showed a relatively high antibacterial activity against *E. coli* and *S. aureus* because the EM/PF127 hydrogels showed very clear inhibitory zones around the specimen. Although the EM/PF127-1, EM/PF127-2, and EM/PF127-3 hydrogels had the same EM contents, the results indicate that the EM/PF127 hydrogels had a different inhibitory zone. The inhibition zones of the EM/PF127-1 hydrogel on *E. coli* and *S. aureus* were 16.1 and 21.6 mm, those of the

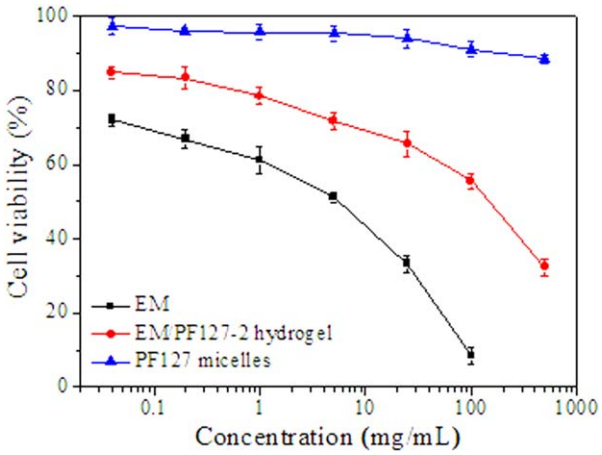

Figure 8. Cytotoxicity of the EM, PF127 micelles, and EM/PF127-2 hydrogels on the L929 cells. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

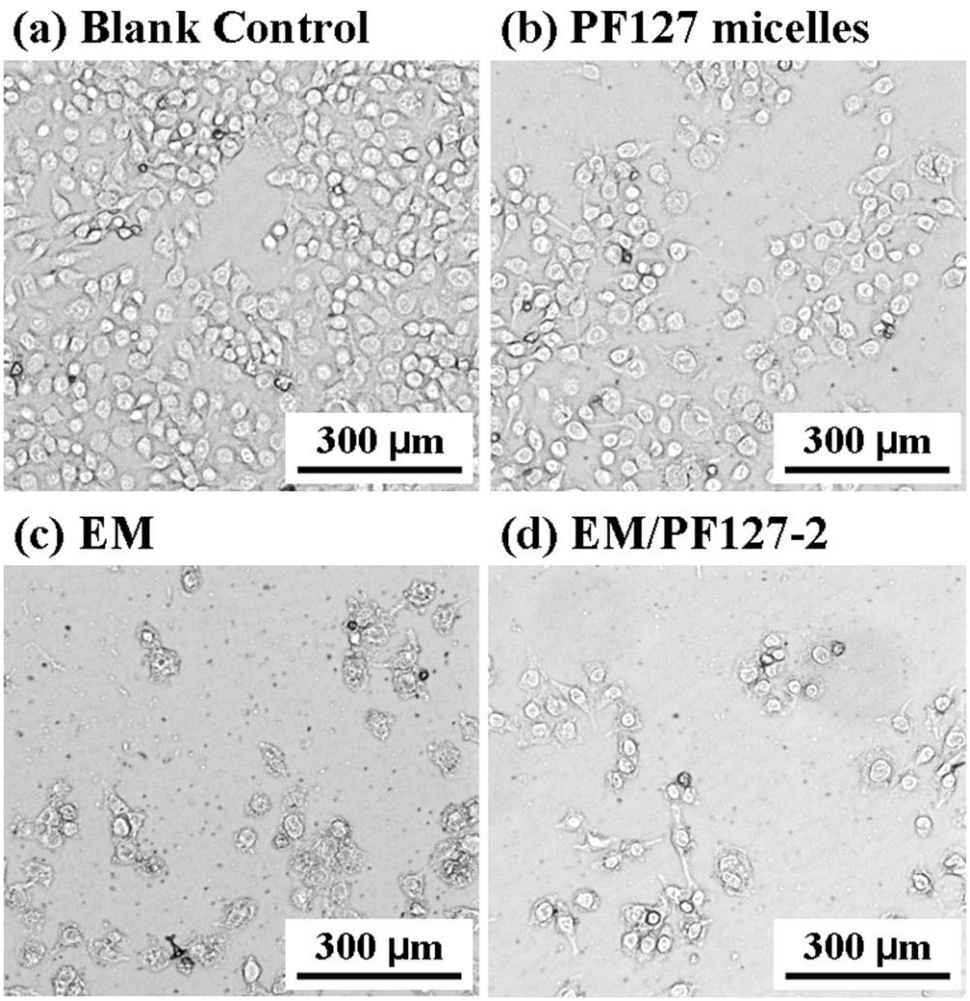

Figure 9. Morphological changes of the L929 cells in the (a) blank control group, (b) PF127 micelles group, (c) EM group, and (d) EM/PF127-2 hydrogel groups.

EM/PF127-2 hydrogel on *E. coli* and *S. aureus* were 14.2 and 17.4 mm, and those of the EM/PF127-3 hydrogel on *E. coli* and *S. aureus* were 11.6 and 12.3 mm, respectively. The diffusion rates of EM from the EM/PF127 hydrogels into agar were different, and the EM release rates decreased with increasing cross-linking time, so the EM/PF127-1 hydrogel had the largest inhibition zones.

Cytotoxicity Test

We also carried out cytotoxicity tests on the EM, PF127 micelles, and EM/PF127-2 hydrogels. When the concentrations of the EM, PF127 micelles, and EM/PF127-2 hydrogels were set at 50 mg/L and after interaction for 24 h, the MTT assays (Figure 8) showed that EM exhibited a serious cytotoxicity to L929 within 24 h of incubation (the cell viabilities of L929 were reduced to 51.1 and 8.4% with TCs of 5 and 100 μg/mL, respectively). The PF127 micelles also showed a slighter cytotoxicity to L929 within 24 h of incubation (the cell viabilities of L929 were reduced to 95.4 and 91% with TCs of 5 and 100 μg/mL, respectively). However, the cell viability of L929 was reduced to 71.1 and 55.5% with EM/PF127-2 hydrogels of 5 and 100 μg/mL, respectively. Therefore, the cytotoxicity of the EM/PF127-2 hydrogel was significantly lower than EM, and the use of the EM/PF127 hydrogels were safer than the direct use of EM, which was in accordance with the result of the inverted-phase contrast microscope measurements (Figure 9). The morphological changes of L929 cells are shown in Figure 9. In the blank control group [Figure 9(a)], the L929 cells had good shapes and presented polygons. The presences of round dividing cells showed their vigorous growth. In the PF127 micelles group [Figure 9(b)], the number of L929 cells decreased. In the EM/PF127-2 hydrogel groups [Figure 9(d)], the L929 cells' shapes became irregular, and the shapes of the majority of the cells were injured. In the EM group [Figure 9(c)], the number of L929 cells decreased significantly, and the shapes of the majority of the cells were seriously injured.

Compared with previous reports,[25–27] we concluded that the EM/PF127 hydrogels were relatively biocompatible nanomaterials with slight cytotoxicity.

CONCLUSIONS

In summary, the PF127 hydrogel was used as the carrier of EM, and an EM/PF127 hydrogel was prepared. The results show that

the release rates of EM decreased with increasing crosslinking time. The EM/PF127 hydrogels had controlled product lifetimes, that is, the ends of antibacterial activities before dissolutions and diffusions of them. The antibacterial effects and inhibitory zones test of the EM/PF127 hydrogels were found to be enhanced with decreasing crosslinking time. The EM/PF127 hydrogels were relatively biocompatible nanomaterials with slight cytotoxicities, so the use of EM would be safer and more efficient. Given these advantages, we expect that the EM/PF127 hydrogels have great potential applications as antibacterial hydrogels in the prevention of postoperative infections.

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