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Poly(2-hydroxyethyl methacrylate) wound dressing containing ciprofloxacin and its drug release studies

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An improved wound dressing with a long-term drug diffusion-efficacy has been developed by UV-radiation technique. It involves incorporation of ciprofloxacin (CIP), at the concentration of 0.5–2.0% (w/v), into a water mixture of 2-hydroxymethacrylate (HEMA) monomer, benzoin isobutyl ether (BIE) initiator and different content of ethylene glycol dimethacrylate (EGDMA) cross-linker. Increasing the concentration of EGDMA would reduce the releasing ratio of CIP from pHEMA. $T_{1/2}$ is increased from 2.64 to 45.67 h when the EGDMA is added from 1 to 8%. In the ranges of $0 \leq F \leq 0.6$, the n value of 1%CIP-pHEMA membranes is increased from 0.48 to 0.81. It indicates that the mechanism of drug release falls between the Fickian and Case II diffusion model. The antibacterial activity of the drug impregnated into the membrane was evaluated by *in vitro* drug kinetic agar plate method. Higher concentration of EGDMA, up to 8% of the cross-linker, extends the drug release. Comparison with the drug-soaked membranes, the newly synthesized 1% CIP-pHEMA membrane (cross-linked with 4% EGDMA) sustains the release of the entrapped drug and maintains the antibacterial activity up to 12 days.

1. Introduction

The skin burn wound infection continues to be a serious of problems in burned patients. Pruitt reported that the bacterial strains isolated from burn wounds changed with time. Initially, Gram-positive staphylococci predominate within 4 hours after burn injury, but they are gradually superseded by Gram-negative pseudomonal species [1–3]. It is also found that the burn wound infection would either delay the healing time or seriously cause death by bacterial sepsis. [4–6]. In order to control the burn wound infection, addition of antibacterial agents to the wound dressing preparations have been shown considerably effective in the reduction of the risk of serious infections.

The poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogels are widely used in the biomaterial field due to its easy oxygen permeability [7], good water transmission and absorption [8, 9], high biocompatibility and non-toxicity [8, 10, 11]. For using in burn wounds, pHEMA wound dressings are proved to prevent excessive fluid loss through open wounds and beneficial with accelerative wound healing capabilities. Unfortunately, it cannot effectively control subsequent microbial invasion at the burn wound sites. In order to prevent the burn wound infection, pHEMA dressings containing drugs, such as silver sulfadiazine had been developed by dipping or pasting, but fast elimination of drugs and lack of a long-term diffusion efficacy remained the drawbacks of these dressings prepared by these techniques [12–16]. Later, some improvements were made by employing the thermal methods for impregnation [17] and immobilization [18, 19] of antibacterial agents onto pHEMA membranes. Kwok and his associates recently incorporated ciprofloxacin (CIP) into pHEMA membrane by employing ultrasonically method and obtained a controlled and discontinuous release of the drug [20]. However, these prepared processes require special techniques and time consuming.

Previously, we have developed an ultra-thin pHEMA dressing with good water transmission and tensile strength [21, 22]. In this study, a simple and rapid UV-radiation technique to incorporate CIP into pHEMA membranes has been developed. The preparation of a wound dressing with a long-term drug diffusion

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efficacy is feasible. The newly developed CIP-pHEMA wound dressing is also evaluated.

2. Experimental

2.1. Materials

2-hydroxyethylmethacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) were purchased from Merck [Darmstadt, Germany] and were purified by distillation under reduced pressure before use. Benzoin isobutyl ether (BIE) was obtained from the TCI [Tokyo, Japan]. Ciprofloxacin·HCl (CIP) was obtained from Serologicals Proteins Inc. [Illinois, USA] and other chemical reagents were purchased from Sigma [San Louis, USA]. De-ionized water was prepared in our laboratory by using reversed-osmosis instrumentals [Millipore, Bedford, MA, USA]. Bacterial strains were collected from the American Type Culture Collection [Rockville, MD, USA] and were grown in Muller-Hinton Medium [Difco, MI, USA] at 37 °C.

2.2. Polymerization by UV-radiation

The details of the polymerization processes were described as before [21, 22]. Briefly, the mixtures of HEMA, EGDMA, BIE (94:5:1), and deionized water (16–20%, w/w) mixed with various amounts of CIP agent, ranging from 0.5 to 2.0% (w/v), were added to fill the mold. Then, the mixture was radiated for 18 min in a UV-reactor [RPR-100, Rayonet, USA]. The wavelength of the UV-radiation is 253.7 nm. Membranes with 0.20–0.75 mm of thickness were obtained. Due to the polymerization of pHEMA hygrogels can be attained conversions about 98.5% degree, so that the CIP loaded pHEMA membranes were used without purification. After fabrication, the products were soaked in distilled water for 5 min, wiped slightly with a sterilized filter paper to suck away the free surface water and punched into discs. Then they were sealed into a plastic bag and maintained at 25 °C, 60% relative humidity.

To assess the stability of CIP under the irradiation conditions used for the polymerization of HEMA membranes, aqueous solutions of CIP were irradiated and the concentration of CIP assayed before and after irradiation. 0.5, 1.0, 1.5, and 2.0% of CIP solution were prepared, and then irradiated as described above. After irradiation, the CIP solutions were measured by high performance liquid chromatography (HPLC) [6000A pump and a 990 photodiode array detector, Waters, Milford, MA, USA]. The stationary phase was HP-ODS (5 μm, 300 $\times$ 4.0 mm i.d.) and the mobile phase was CH_3OH-Na_2HPO_4 = 30:60, pH = 3.0. The flow rate was 1.0 ml/min and the detector was set at 280 nm. A CIP standard curve, ranged from 0.1 to 400 μg/ml, was established and the p-hydroxybenzoic acid was used as an internal standard.

2.3. FT-IR analysis

The IR spectra were measured directly on FT-IR spectrometer [FTS-60, Bio-rad, USA] using 0.2 mm pHEMA films containing different amounts of CIP drug. The spectra were recorded from 4000 to 500 cm^{-1} and 50 scans were made for each sample. The IR spectra of pHEMA films without CIP and 1% CIP in KBr die were measured as the control group.

2.4. Swelling value of the pHEMA without drug

The degree of swelling of the pHEMA membrane containing different EGDMA content was determined in distilled water and in normal saline (0.9% NaCl) solution at 37 °C, respectively. The pHEMA discs were punched to make 0.5 mm thickness and 15 mm diameter and the weight of a dry disc (*Wd*) was first measured with a 4-decimal-point microbalance [AE163, Mettler, Switzerland]. After immersion of the discs in water for 20 h, the equilibrium swelling was reached. The weight of swollen discs were wiped dry with tissue paper and weighted as the wet weight of disc (*Ww*). The equilibrium degree of swelling (q) was obtained according to the Equation 1 as following:

$$q(\%) = 100\% \times (Ww - Wd)/Wd \qquad (1)$$

The result is the average of three measurements for each sample.

2.5. Assay of released CIP drug

CIP-pHEMA membranes were prepared by adding 1% CIP to the different amount of cross-linker (EGDMA: 0, 1, 2, 4, 8 wt% relative to the total amount of HEMA), respectively. Then, 15 mm-diameter discs were punched out from different sections of the 5 $\times$ 10 cm^2 CIP-pHEMA membranes. Each disc was immersed into a brown color bottle containing 2 ml of saline solution at 37 °C. At different time intervals, a 20 μl aliquot was sampled from the medium and the disc was transferred to a new bottle containing 2 ml of saline subsequently. The amount of released CIP was measured by HPLC as previous process. The original amount of the entrapped drug in each disc was obtained by soaking the sample in DMSO/distilled water solution (v/v = 6:4) for 12 h and measured as before.

2.6. *In vitro* antibacterial activity measurement

The *in vitro* tests were performed on the growing cultures of *Pseudomonas aeruginosa* (*Ps.*) and *Staphylococcus aureous* (*St.*) (10 ^{6}cfu/ml) distributed on Mueller-Hinton agar plates. The tested samples were obtained by punctured three 8.0 mm i.d. pHEMA discs entrapped with 1% CIP drug and 4% EGDMA (CIP-M). The negative control disc (B) was prepared by the same method but without containing drug. The pHEMA discs (8.0 mm i.d.) or paper discs (8 mm i.d., Advantec®, Toyo Roshi, Kaisha LTD., Japan) soaked in 1% CIP solution for 3 h were used as the positive control discs (symbol as CIP-S and CIP-D), respectively. Each test disc and the control disc were placed on the agar surface and incubated at 37 °C for 20 h. After incubation, the inhibition zone on the plates was estimated as the efficacy of antibacterial activity.

2.7. Study on drug release kinetics

In drug kinetic release test, the CIP-S, CIP-D, and CIP-M discs were compared by *in vitro* antibacterial zone of inhibition (described as above). All tested discs were peeled off and serially transferred each day for 14 days to another fresh agar plate seeded with *Ps*. The diameter of inhibition zone of each disc was measured and compared to determine drug release kinetics over various time intervals. The minimum inhibitory concentration (MIC) of CIP drug was measured as the threshold of antibacterial activity by using the same agar plate with 0.15 μg/ml of CIP solution.

3. Results and discussion

3.1. Preparation and characterization of CIP-pHEMA wound dressings

In this study, the pHEMA wound dressings incorporated with CIP drug were prepared by using a simple and rapid UV-radiation technique. As the concentration of CIP ranged from 0.5 to 2.0%, the transparent membranes indicate that the drug was homogeneously distributed throughout the matrix. The overall texture of the pHEMA wound dressing containing CIP drug (CIP-pHEMA) was slightly stiff when dry, but it swelled rapidly and turned soft and flexible when it soaked in water. To select CIP as the antibacterial agent for incorporation into the pHEMA wound dressing was based on the following three reasons: (1) CIP is a relatively new synthetic antibiotic exhibit broad-spectrum antibacterial activity, especially against *Ps.* and *St.* strains [23, 24]. (2) The drug has low toxicity and poor absorption from the scar into the blood [25]. (3) The impregnated device typically improves the ability to deliver higher drug concentrations locally at the site of infection. Besides, CIP is relatively stable under heat condition. Table I shows the percentage CIP remaining unchanged after UV-radiation of the aqueous solutions and the CIP-pHEMA membranes. All samples showed a single CIP peak (retention time = 5.27 min) in the chromatograph. No CIP degradation was observed under the condition, and the minimum recovery of unchanged CIP reached 98.15%. These results indicated that CIP was resistant to UV-radiation within 15–25 min and it was, therefore, a good drug candidate for impregnation into pHEMA dressing by employing rapid UV-radiation technique.

TABLE I Percentage of ciprofloxacin remaining unchanged after UV-radiation

	Percentage CIP unchanged (%)*	
CIP concentration (%)	In aqueous solution	In CIP-pHEMA
0.5	98.29 ± 4.73	98.15 ± 5.13
1.0	99.27 ± 2.15	99.14 ± 3.16
1.5	99.67 ± 1.77	99.33 ± 2.19
2.0	98.75 ± 2.08	98.21 ± 2.87

*The data were measured by HPLC and calculated in triplicates.

To analyze CIP entrapped in pHEMA membranes, the FT-IR absorption spectra from 4000 to 500 cm^{-1} are used to measure the samples (Fig. 1). The carboxylic acid of CIP displays a very broad O–H stretching absorption in the region from 3600 to 3150 cm^{-1} and an intensive peak of C=O stretching vibration at 1700 cm^{-1}. However, the C=O peak was assigned overlap with the carbonyl group of pHEMA. In addition, strong peaks near 1637, 1297, and 815 cm^{-1} were indicated the C=C stretching, C–O stretching, and aryl fluoride (C–F) stretching vibration of CIP characteristic absorption, respectively. These bands rise in the absorbed intensity as the amount of CIP increases. The characteristic C–O absorption of pHEMA displays a band near 1151 cm^{-1}. When the drug content reaches to 2.0%, the absorption peak of C–O stretching shifts to 1159 cm^{-1} and the peak becomes broad. The shift of C–O stretching absorption to higher wave number could be explained as follows: at higher concentration of the drug, the distance between CIP molecules and the pHEMA hydroxy group close enough to cause hydrogen bonding formation by interaction of fluorine, carbonyl, and amino group of the CIP with hydrogen atom of pHEMA hydroxy group. Therefore, the absorption peak of C–O of pHEMA becomes weak and broad.

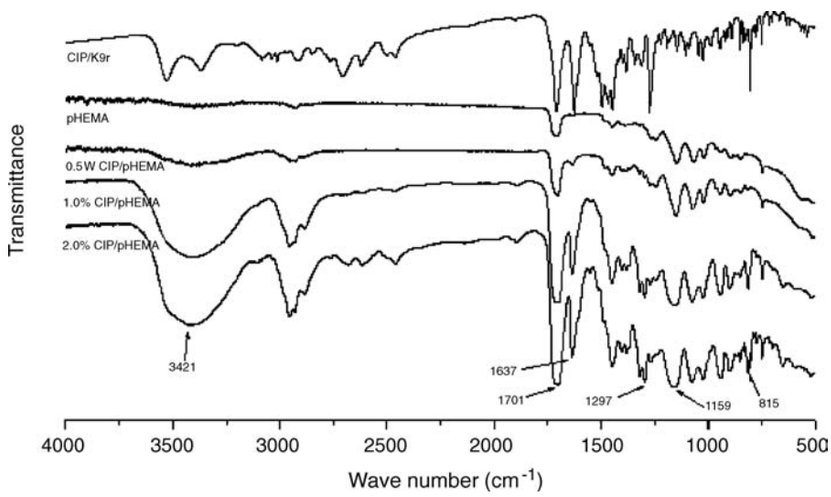

Figure 1 The FT-IR spectra of ciprofloxacin entrapped in pHEMA membranes.

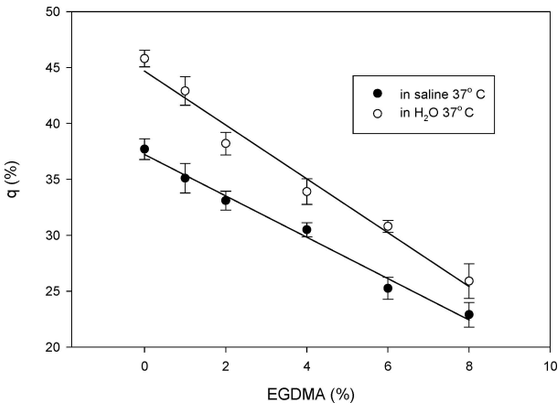

Figure 2 Equilibrium swelling ratio (q%) of pHEMA membranes in saline and aqueous solution as a function of EGDMA cross-linker content.

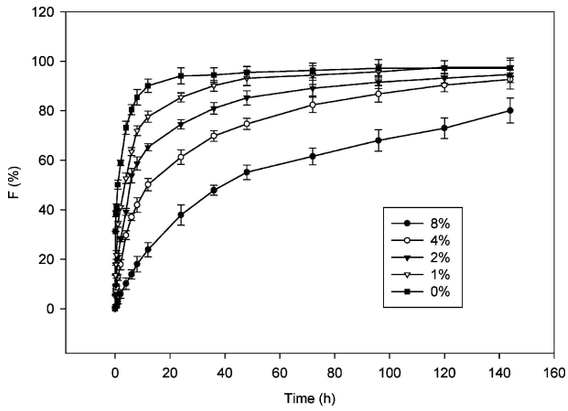

Figure 3 A cumulative profile of released ciprofloxacin from CIP-pHEMA membranes with different EGDMA content ($n = 3$).

3.2. Swelling values of pHEMA

The swelling of pHEMA membranes was examined because it has a significant influence on drug release [15]. In these experiments normal saline solution was employed in order to obtain the behavior of pHEMA dressing in conditions close to the in vivo system. PHEMA discs with 0–8% EGDMA cross-linker were employed and the degree of swelling ratio ($q\%$) was measured after soaked in distilled water or saline for 20 h. The values of $q\%$ are shown in Fig. 2, where a higher of EGDMA content in the polymers results in a lower degree of swelling. It has been reported that an increase of cross-linker EGDMA lead to decrease either water or biological fluids uptake into pHEMA matrices and reduce their swelling values [15, 26, 27]. Our results are consistent with those previous obtained for swelling in water or in saline of pHEMA hydrogels. Therefore, the swelling behavior of the pHEMA dressing will affect on water uptake and the transport of any aqueous soluble drug impregnated in the polymer matrix.

3.3. CIP drug release profile

The CIP drug released from the CIP-pHEMA membranes was carried out in a saline environment and determined by HPLC. The mean cumulative CIP release profile from different concentration of EGDMA ($n = 3$) is plotted as a function of immersion time (Fig. 3). The data indicate that an exponential curve with a rapid release of CIP in the initial 20 h (approximately 60% release) and then a slower rate of release from the same membrane was displayed. The initial burst release of CIP was associated with the rapid transport of water into the initial pHEMA membranes, which formed to porous structure on swollen hydrogels, and push the drug through the membranes quickly. The burst effect became smaller when the concentration of cross-linking EGDMA was increased. As the concentration of EGDMA up to 8%, the 1% CIP-pHEMA would extend the drug release to 128 h. However, after 200 h immersion, greater than 98% of the entrapped CIP was released from all pHEMA membranes. In the previous reported, the effect of cross-linker concentration on the drug release may be interpreted as in term of polymer swelling value and its structural deformation [28–35]. As illustrated in Fig. 2, during the time course of swelling, pHEMA membrane with higher concentration of EGDMA has lower degree of swelling and less water uptake and hence, it reduces the driven force of water to push drugs through the membrane. In other word, EGDMA impedes the drug release mechanism, which prolongs the burst releasing of CIP drug from pHEMA dressings.

3.4. Drug release kinetics

According to the expression derived from Fick's law, drug release from the swelling pHEMA hydrogels can be described by the Equation 2:

$$F = Mt/M_\infty = kt^n \tag{2}$$

where F is the release ratio of drug, Mt is the amount released at time t, M_∞ is the initial drug loading and k and n are drug release constants. It has been reported that $n = 1$ is Case-II release kinetics and $n = 0.5$ corresponds to the Fickian release kinetics. Between these two limiting cases, when n is between 0.5 and 1.0, it is defined as the anomalous release behavior [15, 36–38]. In this work Table II represents the drug release data in the ranges of $0 \leq F \leq 0.6$ for pHEMA membranes containing 1% CIP, in which EGDMA is the cross-linking agent. In the case without any cross-linking

TABLE II The diffusion constant and $T_{1/2}$ of ciprofloxacin from drug loading pHEMA membranes in saline*

EGDMA (%)	n	k	r^2	$T_{1/2}$ (h)
0	0.262 ± 0.042	0.501	0.997	1.01
1	0.480 ± 0.011	0.314	0.991	2.64
2	0.557 ± 0.015	0.208	0.997	4.81
4	0.617 ± 0.027	0.111	0.980	11.45
8	0.814 ± 0.012	0.027	0.985	45.67

*Membranes cross-linked with different content of EGDMA. Experiments performed at 37 °C. Values of n and k are averaged with triplicates.

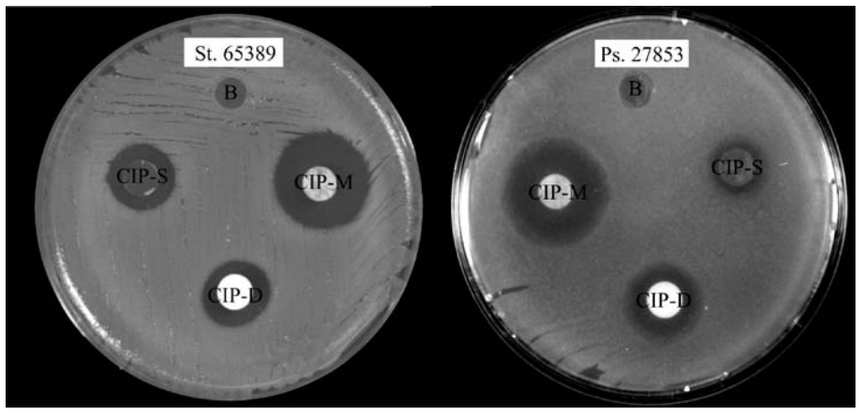

Figure 4 Inhibition zones observed around the CIP-pHEMA discs on agar plate incubated with *St. aureus* and *Ps. aeruginosa* strains.

agent, the CIP-pHEMA membrane has the lowest release index n value (0.262), the largest release constant k (0.501), and the fastest release time $T_{1/2}$ (1.01 h). According to Pepas report [37], the release of drug through the water-filled porous hydrogel should have a release index n smaller than 0.5. In our result $n = 0.262$ for the pHEMA membrane without EGDMA suggested that the entrapped CIP release through a porous water-filled structure and hence, it caused the drug release very quickly. After 2 h immersion, almost CIP was diffused out completely. On the other hand, when the concentration of EGDMA in the hydrogels increased, the duration of CIP release would be sustained for a longer period of time. For example, $T_{1/2}$ is increased to 45.67 h when the EGDMA content reach to 8%. The drug release constant k also depends to the fraction of EGDMA, but it is decreased with an increase in the EGDMA composition. The n values, however, range from 0.48 to 0.81 when the EGDMA increases from 1 to 8% in pHEMA membranes. These results imply that the release mechanism of these CIP-pHEMA membranes falls between the Fickian and Case II diffusion model. According to the physical model of Thomas and Windle [39, 40], the anomalous releasing kinetics is associated with two basic parameters: the diffusion coefficient and the internal stress of pHEMA. As the EGDMA in a lower concentration (1–2%), the CIP drug release is major controlled by diffusion factor. While the EGDMA in a higher concentration (>8%), the CIP release may approach to Case II mechanism ($n \rightarrow 1$) and be controlled by the intrinsic stress of polymer.

3.5. Antibacterial activity of CIP-pHEMA membranes

The antibacterial activity of the 1% CIP-pHEMA membranes cross-linked with 4% EGDMA membrane (CIP-M) was evaluated by the disc diffusion method. Fig. 4 shows the clear inhibitory zone was seen around CIP-M disc in culture plates inoculated with either *Ps.* or *St.* strains at the fourth day. But the other two drug-soaked discs (CIP-S and CIP-D) have less inhibitory zones individually. From the drug kinetic release experiments, the average diameter of inhibition zone of the membranes versus time is continuously measured as shown in Fig. 5. Among those data, CIP-M had significantly exceeded the minimal inhibitory concentration (MIC = 0.15 μg/ml) of CIP for *Ps.* even at 12 days, whereas either CIP-S or CIP-D disc showed a complete and rapid release of drug within 3–5 days. The results indicated that the impregnated CIP drugs were sustained release from the CIP-M membrane and still retained the activity even after UV-radiation.

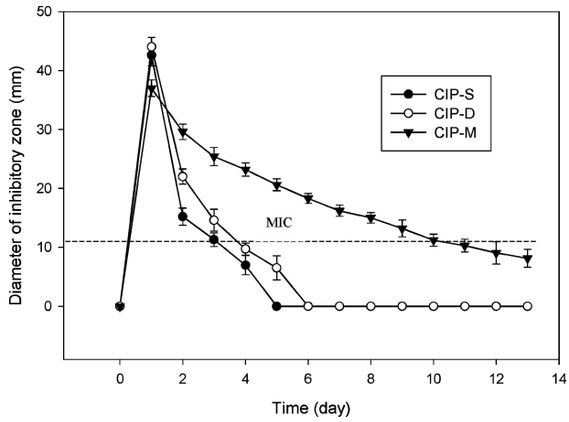

Figure 5 Average inhibitory zones versus time profile for 1% ciprofloxacin soaked or impregnated in pHEMA membranes. Dash line means MIC value ($n = 3$). Test strain: *Ps. aeruginosa.*

4. Conclusion

A simple and rapid technique has been developed to prepare the pHEMA wound dressing containing CIP antibiotics under suitable UV-radiation condition. In drug release experiments, an exponential profile was observed with a rapid release of CIP in the initial 20 h and then a slower rate of release extended to 128 h. Increasing the concentration of EGDMA would reduce the releasing ratio of CIP from pHEMA. In the ranges of $0 \leq F \leq 0.6$, the n value of 1% CIP-pHEMA membranes is ranged from 0.48 to 0.81. It indicates that the mechanism of drug release is controlled by the anomalous releasing kinetics (non-Fickian diffusion). The antibacterial activity of synthesized membrane was evaluated by *in vitro* drug kinetic agar plate method. Comparison with the drug-soaked membranes (CIP-S

or CIP-D), the synthetic CIP-M membrane (with 4% EGDMA) proved to sustain drug release up to 12 days. The study shows that CIP-pHEMA wound dressings overcome the drawback of fast elimination of drugs [12, 16]. Thus, our study provides an alternative way for the preparation of wound dressing with a long-term drug diffusion efficacy.

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