

PHOTO CROSS-LINKED BIODEGRADABLE HYDROGELS FOR ENHANCED VANCOMYCIN LOADING AND SUSTAINED RELEASE*

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Abstract A series of biodegradable hydrogels based on dextran and poly(L-glutamic acid) were fabricated for effective vancomycin loading and release. The preparation of hydrogels was simply achieved by photo cross-linking of methacrylated dextran and poly(L-glutamic acid)-g-hydroxyethyl methacrylate (PGH) in the presence of photoinitiator I2959. The structures of hydrogels were characterized by FTIR and SEM. The swelling and enzymatic degradation behaviors of hydrogels were examined to be dependent on the poly(L-glutamic acid) content in the hydrogels. The higher content of poly(L-glutamic acid) in the gel, the higher swelling ratio and quicker degradation were observed. More interestingly, the hydrogel with higher PGH ratio showed higher vancomycin (VCM) loading content, which might be due to the electrostatic interaction between carboxylate groups in hydrogel and ammonium group of VCM. *In vitro* drug release from the VCM-loaded hydrogels in aqueous solution exhibited sustained release of VCM up to 72 h, while the *in vitro* antibacterial test based on the VCM-loaded hydrogel showed an efficient Methicillin-Resistant *S. aureus* (MRSA) inhibition extending out to 7 days. These results demonstrated that the biodegradable hydrogels which formed by *in situ* photo-cross linking would be promising as scaffolds or coatings for local antibacterial drug release in tissue engineering.

Keywords: Photo cross-linking; Hydrogel; Vancomycin; Antibacterial.

INTRODUCTION

The frequent systemic administrations of antibiotics, that not only eliminate the healthy bacteria but also usually induce antibiotic-resistance, have emerged as a growing problem in antimicrobial treatment^[1, 2]. In response, various innovative drug delivery systems, such as organic/inorganic hybrid scaffolds^[3, 4], surface coatings^[5], wound dressings^[6], polymeric nanoparticles^[7] and LbL multilayer films^[2] *etc.*, have been developed to achieve controlled release and enhanced antibacterial efficacy. Most of the abovementioned strategies take a common advantage to deliver antibiotics locally. By delivering locally, the antibiotics can exert their therapeutic action more effectively, while eliminating the systemic administrations. For this purpose, polymeric hydrogels should be a good choice for their controlled drug loading and release *in situ* in the form of porous scaffolds or surface coatings.

Hydrogels are three-dimensional polymer networks that formed by either chemical or physical cross-linking, which have attracted long-lasting interest for drug delivery and tissue engineering^[8, 9]. Especially, because of the benign aqueous environment in inner hydrogels, hydrogels are usually used as delivery vehicles for loading and controlled release of bioactive molecules such as peptides, nucleic acid and proteins^[10]. In

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addition, environmental responsive polymers also have been incorporated into hydrogels for a variety of controlled drug delivery triggered by temperature, pH or enzyme *etc*^[11, 12]. For example, Chen *et al.*^[13] have prepared thermo- and pH- dual responsive hydrogels for intelligent release of model drug BSA.

Despite of these favorable characters, applications of hydrogels-based delivery systems for antibiotics are still less investigated^[5, 14]. This may be due to the low drug loading quantity and fast drug release in swelling hydrogels, which are far from satisfactory in antibacterial treatment. To address this issue, we present here a facile method to prepare hydrogels with enhanced vancomycin (VCM) loading and sustained release for long-term antibacterial effect. The hydrogels with various dextran and poly(L-glutamic acid) compositions were prepared by directly exposing the polymer solution under UV irradiation. VCM was then loaded into the hydrogels and the drug loading content was increased as the increased weight ratio of poly(L-glutamic acid) in the hydrogels. The *in vitro* drug release in aqueous solution and antibacterial effect were also investigated.

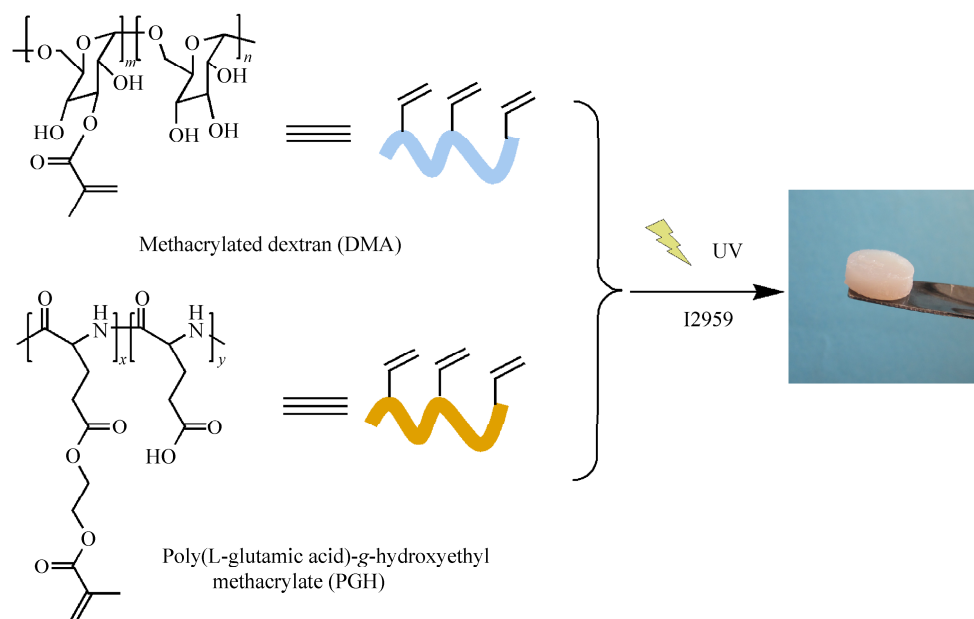
EXPERIMENTAL

Materials

Dextran (from *Leuconostoc* spp., M_r ca. 1.0×10^5 Da) was purchased from Sigma-Aldrich. 2-Hydroxyethyl methacrylate (99%, from J&K Scientific Ltd.) and glycidyl methacrylate (99%, from Adamas Reagent, Ltd.) were used as received. 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (98%, I2959) was obtained from Sigma-Aldrich. Glycidyl methacrylated dextrans (DMA) was synthesized according to the literature^[15]. Synthesis of poly(L-glutamic acid)-g-hydroxyethyl methacrylate (PGH) was described in the previous report (the molecular weight of poly(L-glutamic acid) was determined to be 8.5×10^4 Da by viscometry)^[16]. The grafting ratios of methacrylated side groups of DMA and PGH were about 25 mol% and 29 mol%, respectively, as determined by ¹H-NMR measurements (data not shown).

Preparation of Hydrogels

Hydrogels were prepared by photo cross-linking of DMA and PGH using I2959 as the photoinitiator (Scheme 1). Briefly, DMA was directly dissolved in deionized water with the concentration of $100 \text{ mg} \cdot \text{mL}^{-1}$ (10% in weight percentage); while PGH was first reacted with NaOH in aqueous solution to convert the carboxyl group to sodium carboxylate in the side chain and finally diluted to concentration of $100 \text{ mg} \cdot \text{mL}^{-1}$. Meanwhile, I2959 was also dissolved in the abovementioned stock solutions of DMA and PGH in the concentration of 0.05 wt%. Then,



Scheme 1 Schematic illustration of hydrogels prepared by UV photo cross-linking of DMA and PGH

the DMA and PGH solutions were mixed in the volume ratios of 100:0, 95:5, 90:10 and 85:15 with total volume of 300 μL . The mixed solutions were transferred into a cylindrical tube with a diameter of 1.0 cm and exposed to UV irradiation at 365 nm (*ca.* 4.5 $\text{mW}\cdot\text{cm}^{-2}$) for 10 min to form hydrogels. The obtained samples were subsequently immersed in deionized water for 24 h to reach equilibrium before lyophilization.

Characterization

FTIR spectra were recorded on a Bio-Rad Win-IR instrument using the potassium bromide (KBr) method. The hydrogel samples were pre-crushed into powder before FTIR measurements. The morphologies of the hydrogels were characterized by environmental scanning electron microscopy (ESEM, Micrion FEI PHILIPS). The lyophilized samples were fixed on a SEM specimen holder and sputter-coated with gold before observation.

Swelling Behavior of Hydrogels

The lyophilized hydrogel samples were weighted and immersed in deionized water. At predetermined time intervals, the swelling samples were weighted. The swelling ratio (SR, gram per gram) of the hydrogels was calculated according to the following equation:

$$\text{SR} = (W_t - W_0)/W_0 \quad (1)$$

where W_t and W_0 are the weights of the swollen and dried samples, respectively. All the experiments were carried out in triplicate, and the average values were reported.

Based on the swelling data, the number-average molecular weight between cross linkings (M_c) and cross linking density (ρ_x) can be calculated from Eqs. (2) and (3), respectively^[16].

$$\frac{1}{M_c} = \frac{2}{M_n} - \frac{(\nu/V_1)[\ln(1-\nu_{2,s}) + \nu_{2,s} + \chi\nu_{2,s}^2]}{\nu_{2,r}[(\nu_{2,s}/\nu_{2,r})^{1/3} - 0.5(\nu_{2,s}/\nu_{2,r})]} \quad (2)$$

$$\rho_x = 1/\nu M_c \quad (3)$$

where M_n is the number average molecular weight of dextran (*ca.* $1.0 \times 10^5 \text{ g}\cdot\text{mol}^{-1}$), ν is the partial specific volume of dextran ($0.62 \text{ cm}^3\cdot\text{g}^{-1}$)^[17], V_1 is the molar volume of water ($18 \text{ cm}^3\cdot\text{mol}^{-1}$), χ is the Flory polymer-solvent interaction parameter (0.473 for dextran-water system)^[17], $\nu_{2,r}$ and $\nu_{2,s}$ are the polymer volume fractions before and after swelling, respectively. The theoretical number average molecular weight between cross-linkings ($M_{c, \text{theor}}$) and theoretical cross-linking density ($\rho_{x, \text{theor}}$) were also calculated from Eqs. (4), (5) and (6)^[16, 17], assuming the quantitative reaction of methacrylate pendants to form ideal network.

$$M_{c, \text{theor}} = \frac{M_t \times 100}{\text{DS}} \quad (4)$$

For DMA/PGH hybrid hydrogels

$$M_{c, \text{theor}} (\text{mixed}) = \chi_1 M_{c, \text{theor}} (\text{DMA}) + \chi_2 M_{c, \text{theor}} (\text{PGH}) \quad (5)$$

$$\rho_{x, \text{theor}} = 1/\nu M_{c, \text{theor}} \quad (6)$$

where M_t is the molecular weight of polymer repeating unit, DS is the degree of substitution of methacrylate groups on the polymer chain, χ_1 and χ_2 are the molar fraction of DMA and PGH in the hydrogels, respectively.

In vitro Enzymatic Degradation of Hydrogels

Biodegradation of hydrogels was performed by incubating a small piece of dry sample in 50 mL Tris-HCl buffer solution ($0.1 \text{ mol}\cdot\text{L}^{-1}$, pH 7.4, containing $0.2 \text{ mg}\cdot\text{mL}^{-1}$ Protease K), at 37°C with constant shaking at $75 \text{ r}\cdot\text{min}^{-1}$. At selected time intervals, the samples were taken out, rinsed thoroughly with the buffer solution and weighted. The solution was refreshed twice a day in order to maintain the enzymatic activity. The percentage of residual

sample weight W_r (%) was calculated based on the following equation:

$$W_r (\%) = W_d / W_0 \times 100 \quad (7)$$

where W_0 is the original weight of hydrogels at equilibrium state before degradation test, and W_d is the weight of the swollen sample after degradation at predetermined time.

Drug Loading and Release

The vancomycin (VCM) was loaded into the hydrogel samples by immersing lyophilized hydrogel samples in a 20 mg·mL⁻¹ solution of VCM in 0.01 mol/L PBS solution (pH 7.4) overnight. The VCM-loaded hydrogels were then rinsed with PBS solution for several times and directly transferred into a dialysis bag (MWCO 3500 Da). The rinse solution combined with mother incubation solution was diluted to a certain volume and measured by UV-Vis spectroscopy at 280 nm. The remaining drug content (c_r) could be calculated by using a standard curve method. Thus, the drug loading content (DLC) in the hydrogels was calculated according to the equation:

$$\text{DLC (wt\%)} = (c_0 - c_r) / [(c_0 - c_r) + W_H] \times 100 \quad (8)$$

where c_0 is the amount of VCM in the initial incubating solution, c_r is the remaining amount of drug in the solution after drug loading, and W_H is the weight of dried hydrogel samples.

The drug release test was initiated by placing the end-sealed dialysis bag into 25.0 mL release medium (0.01 mol·L⁻¹ PBS, pH 7.4) at 37 °C with continuous shake at 75 r·min⁻¹. At different time intervals, 1.0 mL of the release medium was taken out and 1.0 mL of fresh PBS solution was replenished. The amount of released VCM was determined by UV-Vis spectroscopy at 280 nm.

In vitro Antibacterials Activity

A clinical isolate of Methicillin-Resistant *S. aureus* (MRSA), termed EDCC 5055 (Culture Collection, Institute of Medical Microbiology, China-Japan Hospital Changchun, China) was obtained from a patient with wound infection. It was diluted in broth and plated on a blood agar plate. This plate was left in an incubator at 37 °C for 24 h. The identity of the organism was then checked with an antibiogram (Dade Microscan Walkaway96, Siemens, Germany) and made ready for experiment. In order to evaluate the antimicrobial activities of the materials *in vitro*, a colony of Methicillin-Resistant *S. aureus* (MRSA) was put in saline and prepared for bacterial fluid of 0.5 Mcintosh concentrations, and thus spread on M-H agar plate. The VCM-loaded hydrogel was placed in diagnostic sensitivity test medium, incubated at 37 °C for 24 h and the diameter of inhibition zone was recorded. The drug-free hydrogel sample was also loaded on the same plate as the negative control. The M-H agar plate was replaced every day and reloaded with the same VCM-loaded and VCM-free hydrogel samples; the diameter of inhibition zone was monitored in every 24 h until it was close to the diameter of hydrogel (*ca.* 1.0 cm).

RESULTS AND DISCUSSION

Preparation and Characterization of Hydrogels

The hydrogels were conveniently synthesized *via* cross-linking of methacrylated dextran (DMA) and poly(L-glutamic acid)-g-hydroxyethyl methacrylate (PGH) by exposing their mixed polymer solution under UV irradiation for *ca.* 10 min. Hydrogels containing different DMA and PGH weight ratios were synthesized, and the details are given in Table 1. I2959 photo-initiator has been proved to be biocompatible and is widely used in the biomedical applications. The formed hydrogels were characterized by FTIR spectra (Fig. 1). The disappearance of wagging —C=C—H at 950 cm⁻¹ confirmed the successfully photo-induced radical cross linking of double bond between DMA and PGH^[18]. Meanwhile, the —O—H strengthening and —C—O— bending peaks at 3430 and 1021 cm⁻¹, respectively, further verified the incorporation of dextran in the hydrogels. The morphologies of hydrogels were further characterized by SEM observation. As shown in Fig. 2, the as-prepared hydrogels are cylinder scaffolds (Fig. 2, inset) with typical porous structures, which may be suitable for drug loading and release. Furthermore, the pore size of Gel-3 is observed to be larger than that of

Gel-1, which should be due to the higher swelling ratio of Gel-3 relative to that of Gel-1 that will be discussed in the next part.

Table 1. Synthesis and characterizations of hydrogels

Hydrogels	V_{DMA}^a (μL)	V_{PGH}^b (μL)	M_c ($\text{g}\cdot\text{mol}^{-1}$)	ρ_x ($\text{mmol}\cdot\text{cm}^{-3}$)	$M_{c,\text{theor}}$ ($\text{g}\cdot\text{mol}^{-1}$)	$\rho_{x,\text{theor}}$ ($\text{mmol}\cdot\text{cm}^{-3}$)	W_{VCM}^c (mg)	DLC (%)
Gel-1	300	0	3462.0	0.466	648.0	2.489	7.4	19.8
Gel-2	270	30	—	—	624.5	2.583	9.0	23.1
Gel-3	240	60	—	—	601.8	2.680	11.4	27.5
Gel-4	210	90	—	—	579.9	2.781	13.7	31.4

^a Volume of DMA solution added (10 wt% in deionized water with 0.05 wt% I2959);

^b Volume of PGH solution added (10 wt% in deionized water with 0.05 wt% I2959);

^c Amount of drug in the hydrogels

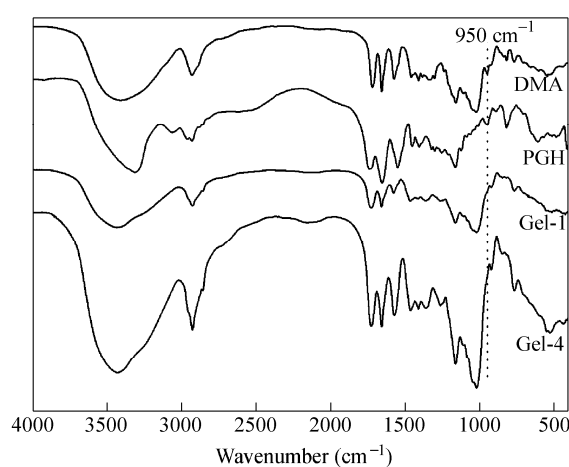


Fig. 1 FTIR spectra of DMA, PGH, Gel-1 and Gel-4

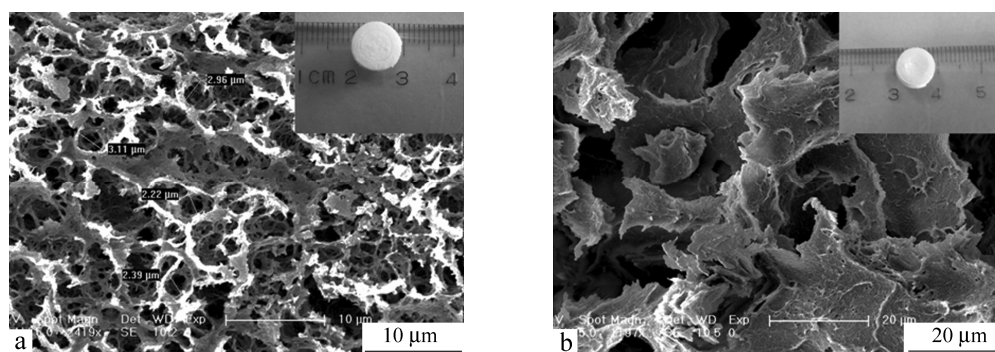


Fig. 2 Typical SEM images of (a) Gel-1 and (b) Gel-3, inset: photography images of Gel-1 and Gel-3

Swelling Behavior of Hydrogels

The swelling behavior of hydrogels was studied by immersing the dried hydrogel samples in deionized water and the results are showed in Fig. 3. All the samples showed a quick taking up of water in less than 15 min and reach equilibrium after about 30 min, indicating the hydrophilic nature and porous structure of the samples. Moreover, the equilibrium swelling ratio increased as the increase of PGH in the hydrogels, which may be resulted from the more hydrophilic poly(L-glutamic acid) sodium compared to dextran or possible decrease of cross linking density in the hydrogels. To reveal the real cause of increasing equilibrium swelling ratio after addition of PGH polymer, the number average molecular weight (M_c) and cross linking density (ρ_x) were calculated both experimentally and theoretically. As listed in Table 1, the experimental M_c and ρ_x of Gel-1 are $3462.0 \text{ g}\cdot\text{mol}^{-1}$

and $0.466 \text{ mmol}\cdot\text{cm}^{-3}$, respectively, which are obviously different from the theoretical results ($M_{c,\text{theor}}$ and $\rho_{x,\text{theor}}$ of Gel-1 are $648.0 \text{ g}\cdot\text{mol}^{-1}$ and $2.489 \text{ mmol}\cdot\text{cm}^{-3}$, respectively). The lower ρ_x value than $\rho_{x,\text{theor}}$ indicated that the hydrogel was formed mainly by intramolecular cross-linkings, and there were still some un-reacted methacrylate groups existed in the hydrogel (though it is hard to be observed in the FTIR measurement in Fig. 1)^[17]. Unfortunately, the experimental M_c and ρ_x of Gel-2, Gel-3 and Gel-4 can not be calculated by the Eqs. (2) and (3), since the existence of two component polymers in the hydrogels. Nevertheless, from the theoretical calculation (Table 1), it is realized that the cross-linking density should be slightly increased as the increase of PGH amount in the hydrogels, if assuming the same reactivity of the methacrylate side groups in DMA and PGH. Therefore, we can conclude that the increasing swelling ratio from Gel-1 to Gel-4 should be mainly ascribed to the addition of more hydrophilic poly(L-glutamic acid) sodium in the hydrogel.

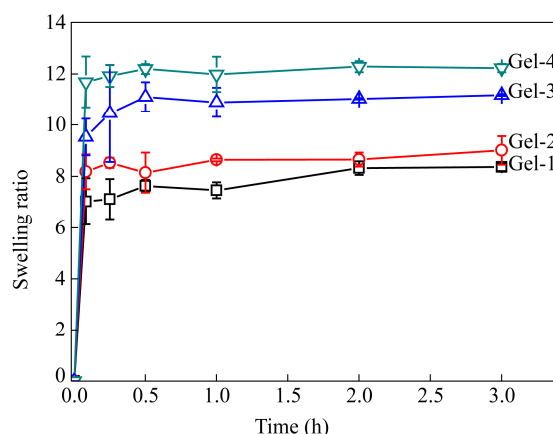


Fig. 3 The swelling behavior of hydrogels (All the results are based on $n = 3$ measurements.)

Enzymatic Degradation of Hydrogels

It has been reported that Protease K can cause degradation of both polypeptides and polyesters effectively^[13, 19]. In this study, the degradation of hydrogels in the presence of $0.2 \text{ mol}\cdot\text{L}^{-1}$ Protease K was performed in PBS pH 7.4, and the results are shown in Fig. 4. The hydrogel without PGH showed no remarkable degradation during the test. This may be because of the low swelling ratio of Gel-1 that prevents the protease to penetrate into hydrogel and induce degradation of ester linker. On the other hand, all the hydrogels containing PGH

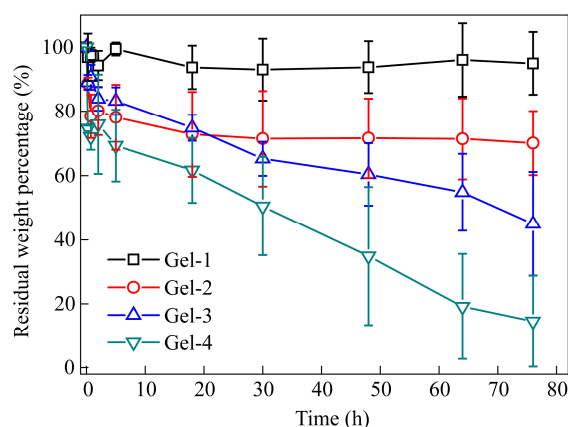


Fig. 4 Enzymatic degradation of hydrogels in $0.1 \text{ mol}\cdot\text{L}^{-1}$ Tris-HCl buffer solution (pH 7.4) containing $0.2 \text{ mg}\cdot\text{mL}^{-1}$ Protease K (All the results are based on $n = 3$ measurements.)

showed notable degradation behavior and the more degradation was observed as weight percent of PGH increased. The residual weight percentages after degradation for 76 h were 70.1 wt%, 45.0 wt% and 14.9 wt% for hydrogels containing 10 wt%, 20 wt% and 30 wt% PGH, respectively. The improved degradation behavior should be ascribed to the increasing swelling ratio that may allow more protease to enter the hydrogel; and the presence of more poly(L-glutamic acid) which is degraded by polymer chain secession. In general, the biodegradability of the hydrogels demonstrates the promising use of these materials for *in vivo* applications.

In vitro Drug Loading and Release

The loading of VCM into hydrogels was achieved by directly immersing the dried hydrogel sample into VCM solution in PBS ($20 \text{ mg} \cdot \text{mL}^{-1}$). The drug loading content (DLC) was measured and listed in Table 1. It is interesting to note that the DLC increased dramatically from 19.8% to 31.4% as the PGH content increase from 0 wt% to 30 wt%. And the drug amount loaded in Gel-4 was about 2 folds of that in Gel-1. The distinct DLC was basically resulted from the different polymer compositions in hydrogels. For Gel-1 containing 100% dextran, the loading of VCM was mainly driven by the swelling of hydrogel, so the resultant drug amount (7.4 mg) in the hydrogel was close to that of the VCM stock solution at the volume of swelling hydrogel ($20 \times 0.3 = 6.0 \text{ mg}$). In contrast, the loading of VCM in Gel 2–4 that contain different poly(L-glutamic acid) weight ratios should be ascribed to both swelling and the electrostatic interaction between carboxylate groups in poly(L-glutamic acid) and ammonium group of VCM. Thus, we conclude that VCM can be loaded into the hydrogel in high content by introducing a small amount of polyanion, such as poly(L-glutamic acid).

The *in vitro* drug release was then conducted in 0.01 mol/L PBS ($\text{pH } 7.4$), and the results are shown in Fig. 5. All the hydrogels exhibited a quick release in the first 7 h, ascribing to the quick diffusion of VCM drug that was free dissolved in the hydrogels. However, the release rate was observed to be decreased as the increase of PGH in the hydrogel with sustained release of VCM for *ca.* 72 h for Gel 2–4 and *ca.* 48 h for Gel-1. This may be because that there was no interaction between VCM and dextran scaffold, resulting in fast diffusion of VCM drug into release medium from Gel-1. For Gel 2–4, the release of VCM was relatively slow while exhibiting more sustainable release pattern, which should be due to the electrostatic interaction between the positive VCM and negative hydrogel scaffold. In this case, the drug release was not only caused by drug diffusion, but also caused by ion exchange induced release of VCM that electrostatically bonded in the gel scaffold, which thus lowering the drug release rate and extending the drug release time. It also should be noted that though the release patterns in Fig. 5 were almost the same for Gel-3 and Gel-4, the corresponding released drug amount from Gel-4 was higher than that from Gel-3, because of the higher DLC of Gel-4 as compared with Gel-3. Therefore, the introduce of poly(L-glutamic acid) can endow the hydrogels with both enhanced drug loading content and more sustainable release ability.

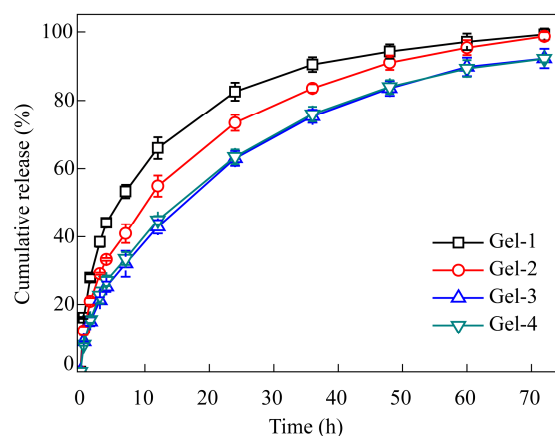


Fig. 5 *In vitro* drug release profiles of hydrogels in $0.01 \text{ mol} \cdot \text{L}^{-1}$ PBS, $\text{pH } 7.4$. Data are presented as mean $\pm$ standard deviation ($n = 3$).

In vitro Antibacterial Activity

For *in vitro* antibacterial test, Gel-3 with relative high VCM loading content (27.5 %) and potential sustainable release ability was selected for the proof-of-concept study. As shown in Fig. 6, the VCM-loaded Gel-3 sample showed effective antibacterial function against MRSA with inhibition zone gradually decreased from 29 mm in the first day to 11 mm in the seventh day, demonstrating the sustained release of VCM from hydrogel during the test duration. The even prolonged VCM release time in antibacterial test may be because of the limited diffusion of VCM drug in diagnostic sensitivity test medium. However, the control hydrogel without VCM loaded exhibited no antibacterial activity and the MRSA can proliferate on the surface of hydrogel (Fig. 6c). These results indicate the potential use of the as-prepared hydrogels for clinical antibacterial treatment.

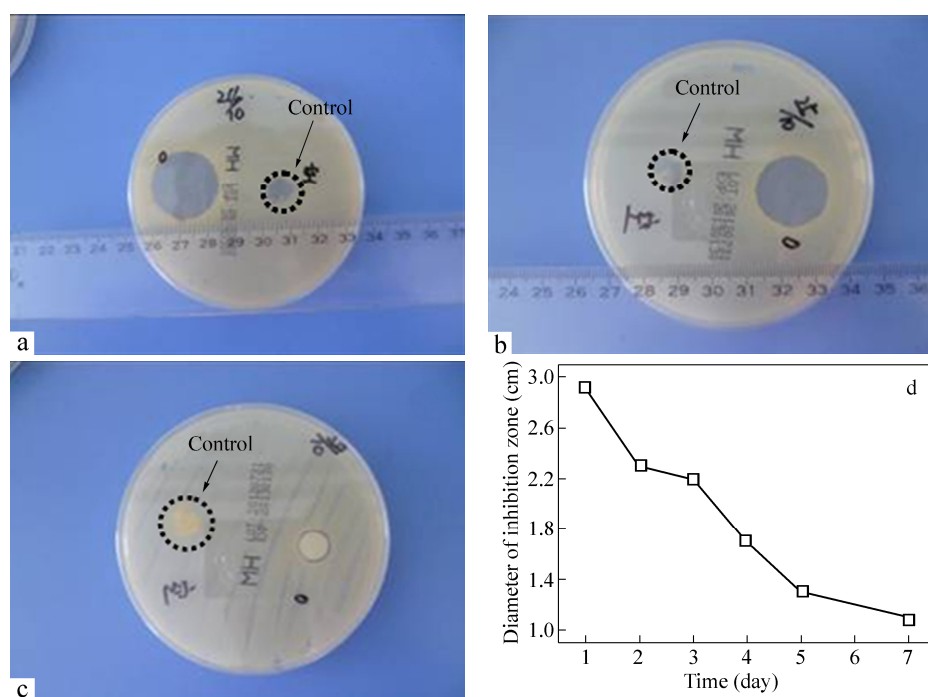


Fig. 6 *In vitro* antibacterials assay showing inhibition of MRSA by VCM-loaded Gel-3 in agar plate at (a) 2nd day, (b) 3rd day and (c) 7th day; (d) Diameter of inhibition zone of MRSA in agar plate at different days

CONCLUSIONS

A series of biodegradable hydrogels with different dextran and poly(L-glutamic acid) weight ratios were conveniently prepared by photo cross-linking in the presence of photo-initiator I2959. The formation of hydrogels was verified by FTIR and SEM measurements. The swelling and enzymatic degradation behaviors of the hydrogels were tested to be dependent on the composition of hydrogels. The higher poly(L-glutamic acid) content in the hydrogels would lead to higher swelling ratio and quicker enzymatic degradation. *In vitro* drug release in aqueous solution revealed the sustained drug release for a period of 72 h. Moreover, the *in vitro* antibacterial test showed an efficient MRSA inhibition extending out to 7 days. Therefore, these results suggested that the photo cross-linking hydrogels could be promising as scaffolds or coatings for local antibacterial drug release in tissue engineering.

REFERENCES

- 1 Liu, S.Q., Yang, C., Huang, Y., Ding, X., Li, Y., Fan, W.M., Hedrick, J.L. and Yang, Y.Y., *Adv. Mater.*, 2012, 24(48): 6484
- 2 Shukla, A., Fang, J.C., Puranam, S. and Hammond, P.T., *J. Control Release*, 2012, 157(1): 64
- 3 Zhang, J.Z., Wang, C.Y., Wang, J.C., Qu, Y. and Liu, G.Y., *Drug. Deliv.*, 2012, 19(5): 264
- 4 Shi, X., Wang, Y., Ren, L., Zhao, N., Gong, Y. and Wang, D.A., *Acta Biomater.*, 2009, 5(5): 1697
- 5 Zhang, L.F., Yang, D.J., Chen, H.C., Sun, R., Xu, L., Xiong, Z.C., Govender, T. and Xiong, C.D., *Int. J. Pharm.*, 2008, 353(1-2): 74
- 6 Zilberman, M. and Elsner, J.J., *J. Control Release*, 2008, 130(3): 202
- 7 Xiong, M.H., Li, Y.J., Bao, Y., Yang, X.Z., Hu, B. and Wang, J., *Adv. Mater.*, 2012, 24(46): 6175
- 8 Chen, Y.M., Yang, J.J., Osada, Y. and Gong, J.P., *Chinese J. Polym. Sci.*, 2011, 29(1): 23
- 9 Peppas, N.A., Hilt, J.Z., Khademhosseini, A. and Langer, R., *Adv. Mater.*, 2006, 18(11): 1345
- 10 Vermonden, T., Censi, R. and Hennink, W.E., *Chem. Rev.*, 2012, 112(5): 2853
- 11 Gao, X.Y., He, C.L., Zhuang, X.L., Zhao, C.W., Xiao, C.S. and Chen, X.S., *Acta Polymerica Sinica (in Chinese)*, 2011, (8): 883
- 12 Xiao, C.S., Tian, H.Y., Zhuang, X.L., Chen, X.S. and Jing, X.B., *Sci. China, Ser. B*, 2009, 52(2): 117
- 13 Zhao, C.W., Zhuang, X.L., He, P., Xiao, C.S., He, C.L., Sun, J.R., Chen, X.S. and Jing, X.B., *Polymer*, 2009, 50(18): 4308
- 14 Zhang, X., Soontornworajit, B., Zhang, Z., Chen, N. and Wang, Y., *Biomacromolecules*, 2012, 13(7): 2202
- 15 van Dijk-Wolthuis, W.N.E., Kettenes-van den Bosch, J.J., van der Kerk-van Hoof, A. and Hennink, W.E., *Macromolecules*, 1997, 30(11): 3411
- 16 Peppas, N.A., Moynohan, H.J. and Lucht, L.M., *J. Biomed. Mater. Res.*, 1985, 19(4): 397
- 17 Ferreira, L., Gil, M.H. and Dordick, J.S., *Biomaterials*, 2002, 23(19): 3957
- 18 Zhao, C.W., He, P., Xiao, C.S., Gao, X.Y., Zhuang, X.L. and Chen, X.S., *J. Appl. Polym. Sci.*, 2012, 123(5): 2923
- 19 Zeng, J., Chen, X.S., Liang, Q.Z., Xu, X.L. and Jing, X.B., *Macrom. Biosci.*, 2004, 4(12): 1118