Poly(ethylene) glycol hydrogel based on oxa-Michael reaction: Precursor synthesis and hydrogel formation

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This paper reported a facile strategy for the one-pot synthesis of vinyl sulfone (VS) group terminated hydrogel precursors [poly(ethylene) glycol (PEG)-VS] and PEG hydrogels via catalytic oxa-Michael reaction. Nine potential catalysts were investigated for the reaction between PEG and divinyl sulfone, among which 4-dimethylaminopyridine (DMAP) prevailed for its high catalytic activity. DMAP produced PEG-VS with a conversion of more than 90% in 2 h under a solvent-free condition at room temperature, which significantly simplifies the synthesis of PEG-VS. The preparation of PEG hydrogels was realized by adding glycerol as a crosslinker, and the physical and the mechanical properties were easily controlled by changing the crosslinker concentration as well as the PEG chain length. This strategy can also be applied to other polyhydroxy compounds as crosslinkers, and thus, a library of hydrogels with designed structures and desired properties could be prepared. The PEG hydrogels showed good antifouling properties, low cytotoxicity, and ability to release drugs at a tunable rate, indicating versatile potential bioapplications. © 2017 American Vacuum Society.

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I. INTRODUCTION

Poly(ethylene) glycol (PEG) hydrogels are of great interest in tissue engineering,^{1–3} diagnostics,^{4–6} and drug delivery^{7–9} because of their good biocompatibility, high permeability to water and ions, and tunable mechanical properties.¹⁰ To date, many strategies have been reported for PEG hydrogel preparation, with those based on the Michael-type reaction topping the list due to its rapid reaction under mild conditions.^{11–14} To be incorporated into a hydrogel network, PEGs bearing chemically more reactive groups [e.g., acrylate, vinyl sulfone (VS), etc.] are often utilized as precursors because of the chemical inertness of hydroxyl groups.^{15–17} Among such precursors, vinyl sulfone-terminated PEGs (PEG-VS) have received great attention because they are selective for the reaction with thiol groups^{18–20} under neutral conditions and able to react with amine groups in basic solutions.^{21–23}

The widespread applications of PEG-VS based hydrogels^{24–26} are facilitated by the development of various methods for PEG-VS synthesis. However, these methods involve either a lengthy multiple-step reaction routine^{27,28} or PEG reactions with divinyl sulfone (DVS) under harsh basic conditions.^{29–31} The former contains several steps of reaction in aqueous solutions, resulting in complex purification steps and low yields. The latter requires a stringent anhydrous condition and inert gas protection, and it usually takes a few days to achieve high conversions. Although sodium hydroxide has been shown to promote the PEG-DVS addition reaction in the aqueous solution,³² the potential hydrolysis reaction³³ may limit the degree of modification. The complexity in the synthesis of PEG-VS thus impedes the synthesis of PEG hydrogels.

Recently, Strasser and Slugovc reported that organic nucleophiles could enable the reactions between DVS and alcohols under mild conditions.³⁴ It thus brings new options to prepare PEG-VS and PEG hydrogels. Herein, we investigated the application of the catalytic oxa-Michael reaction for facile, one-pot synthesis of PEG-VS with a high yield.

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Nine potential catalysts were investigated for the reaction between PEG and DVS. The reaction mechanism was proposed, and the effect of solvents was quantitatively studied. By adding glycerol as a crosslinker and varying its concentrations, PEG hydrogels with tunable physical and mechanical properties were prepared. The ability of the hydrogels to resist nonspecific protein adsorption and their cytotoxicity were assessed. Finally, the applicability of the hydrogels for drug release was demonstrated using Nile Red and Oleanic acid as model drugs.

II. EXPERIMENT

A. Chemicals

Triethylene glycol monomethyl ether (M-EG₃-OH), 4-dimethylaminopyridine (DMAP), 1-methylimidazole (MIM), 1,4-diazabicyclo[2.2.2]octane (DABCO), 3,5-pyridinedicarboxylic acid (PDA), tris(3-sulfophenyl)phosphine trisodium salt (TPPTS), triphenylphosphine (PPh₃), tris(2-carboxyethyl)phosphine (TCEP), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), cysteine, Nile Red, and Oleanic acid were purchased from Aladdin Industries Co Ltd. (Shanghai, China). Triethylamine (NEt₃) and pyridine were purchased from Guangfu Fine Chemical Research Institute (Tianjin, China). DVS was obtained from Xiya Chemical Ltd. (Shandong, China). Hexaethylene glycol (EG₆) and polyethylene glycol (Mw = 2000 and 4000) were purchased from TCI Co Ltd. (Tokyo, Japan). Thiazolyl blue tetrazolium bromide (MTT) was purchased from Aldrich (St. Louis, MO, USA). Fluorescently labeled bovine serum albumin (FITC-BSA) was purchased from Beijing Biosynthesis Biotechnology Co Ltd. (Beijing, China). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Thermo Scientific (Hyclone TM, Logan, UT).

B. Synthesis of a hydrogel precursor

Hexaethylene glycol of 560 mg (EG₆, 2 mmol) was mixed with 1.9 g of DVS (16 mmol, 8 eq.), followed by the addition of 24 mg of DMAP (0.2 mmol, 0.1 eq.). This mixture was stirred at room temperature for 2 h. The product was purified with a silica gel column, affording VS-EG₆-VS (721 mg, 91.0%). ¹H NMR (500 MHz, CDCl₃): δ 6.80 (dd, 2 H, SO₂CH=CH₂), 6.41 and 6.11 (dd, 4 H, SO₂CH=CH₂), 3.90 (t, 4 H, SO₂CH₂CH₂), 3.26 (t, 4 H, SO₂CH₂CH₂), 3.64 (m, 24 H, others CH₂).

C. Screening of catalysts

M-EG₃-OH of 330 mg was mixed with 950 mg of DVS (4 eq.), and 0.1 eq. of catalyst was added. This mixture was stirred at room temperature for 2 h. The unreacted DVS was removed with a silica gel column, and the reaction conversion was determined by ¹H NMR.

D. Study of the reaction mechanism

DVS of 118 mg was dissolved in 0.5 ml of deuterated methanol (>10 eq.), and 12 mg of DMAP (0.1 eq.) was added. After reacting for 15 min at room temperature, the ¹H NMR measurement was conducted.

E. Solvent effect

M-EG₃-OH of 330 mg and 950 mg of DVS (4 eq.) were dissolved in 4 ml of solvent, and 24 mg of DMAP (0.1 eq.) was added. This mixture was stirred at room temperature for 12 h. The unreacted DVS was removed with a gel column, and the conversion was determined by ¹H NMR.

F. Hydrogel formation and characterization

Using the ratio of glycerol to EG₆ of 2:1 as an example, 180 mg of glycerol (2 mmol), 300 mg of EG₆ (1 mmol), and 500 mg of DVS (4 mmol) were mixed, 50 mg of DMAP (0.4 mmol) was added, and this mixture was left to react at room temperature for 2 h. The gel was then washed with ethanol to remove any unreacted small molecules and subsequently hydrated in water to prepare hydrogels.

To determine the degree of crosslinking and the water content of the hydrogels, the freshly prepared gel was washed with ethanol three times and subsequently dried in a vacuum oven. The degree of crosslinking was determined by the mass ratio of the dried gel to the raw materials. Then, the dried hydrogel was fully hydrated in water, and the mass of imbibed water was measured. The water content was determined by the mass ratio of the absorbed water to the dried gel [Eq. (1)]

$$\text{Water content} = (m_h - m_d)/m_d, \quad (1)$$

where m_h is the mass of fully hydrated hydrogels and m_d is the mass of dried hydrogels.

The amount of residual vinyl sulfone group of the gel was determined using Ellman's test.³⁵ 25 mg sample of fully hydrated hydrogel was immersed in 200 μ l of cysteine solution (1 mM, pH=8.0) and incubated at 25 °C for 4 h. Afterward, 250 μ l of the reaction solution and 50 μ l of Ellman's agent solution (20 mg of DTNB dissolved in 5 mL of reaction buffer) were added to 2.5 mL of reaction buffer and incubated for 15 min at 25 °C. The absorbance was determined at 412 nm using a UV-visible spectrophotometer, and the amount of reacted cysteine was calculated. The amount of residual vinyl sulfone group in the hydrogel sample was equal to that of the reacted cysteine.

Rheological monitoring of gelation was performed using the parallel plates and the oscillatory testing mode using a MCR 302 Rheometer (Anton Paar GmbH, Austria). The raw materials and the catalyst were well mixed, 600 μ l of the mixture was poured on the lower plate, and then the upper plate was set at a distance of 1 mm above the lower plate. An angular frequency of 1 Hz and a deformation amplitude $r^\circ = 0.01$ were selected to ensure that the dynamic oscillatory deformation was within the linear regime. The storage

modulus of the as-prepared gel was monitored at 25 °C using the time sweep mode. Afterward, the gel was washed with ethanol three times, 200 μ l of water was subsequently poured on the as-prepared gel, and the storage modulus was monitored for up to 30 min of hydration. Error bars represent the standard deviations of the mean ($n = 3$).

G. Protein adsorption

Solutions of FITC-BSA were prepared at the concentration of 10, 25, 50, 100, 250, and 500 μ g/ml. A calibration curve was prepared to correlate the concentration of FITC-BSA with the fluorescence intensity with $\lambda_{ex} = 495$ nm and $\lambda_{em} = 525$ nm. Fully hydrated hydrogels of 25 mg were incubated in 100 μ l of FITC-BSA solutions (500 μ g/ml) for 2 and 24 h. The protein adsorption was determined from the difference in fluorescence intensity between the original solution and the solution after hydrogel incubation. Error bars represent the standard deviations of the mean ($n = 4$).

H. *In vitro* cytotoxicity of PEG hydrogels

The cytotoxicity of PEG hydrogels prepared with different ratios of glycerol to EG₆ was assessed using direct contact assay.³⁶ Briefly, bone marrow stromal cells were seeded on a 24-well polystyrene tissue culture plate at a density of 50 000 cells per well and incubated for 24 h at 37 °C in DMEM supplemented with FBS (10%) and penicillin/streptomycin (1%). The sterilized PEG hydrogels were subsequently placed on top of the cells in the experimental wells with the contact area being more than 50%. For reference, wells without hydrogel treatment were set as negative control and wells treated with 0.64% phenol were set as positive control.³⁷ Following another 24 h of incubation at 37 °C, the amounts of living cells were determined using the MTT test. Error bars represent the standard deviations of the mean ($n = 3$).

I. Drug release

The PEG hydrogel of 150 mg was incubated in 2 ml of Nile Red/Oleic acid solution (90 μ g/ml in acetonitrile) at 37 °C for 12 h, followed by washing thrice with acetonitrile. The amount of residual Nile Red was determined using a fluorescence spectrophotometer with $\lambda_{ex} = 540$ nm and $\lambda_{em} = 650$ nm, and the amount of residual Nile Red was determined using high performance liquid Chromatography. The encapsulation efficiency was calculated according to a standard curve. The drug-loaded PEG hydrogel was subsequently incubated in 1 ml of water at 37 °C. The released drug was collected every 24 h and dissolved with acetonitrile (containing 20% water) for further quantification.

III. RESULTS AND DISCUSSION

A. Synthesis of a hydrogel precursor

PEG-VS is a kind of commonly used hydrogel precursor. Traditionally, the synthesis of PEG-VS involves a multiple step reaction routine,²⁷ which includes several steps of

aqueous reactions. Complex purification steps were required because of the excellent solubility of PEG in both water and organic solvents. The procedure takes more than three days to achieve PEG-VS with a low yield. The reaction of PEG with DVS under harsh basic conditions, e.g., NaH, can also produce PEG-VS.¹³ The stringent anhydrous condition is required to prevent the base from hydrolysis. Several days of reaction under inert gas protection is usually needed to achieve a high yield.

Herein, nitrogen/phosphor containing organic base was employed to promote the oxa-Michael reaction between PEG and DVS, providing a facile strategy for the synthesis of PEG-VS, as shown in Scheme 1. Compared to the multi-step reaction strategy, this method significantly simplifies the synthetic operation and shortens the reaction time. At the same time, the physical and chemical stabilities of the organic base showed great superiority over traditional alkalis. Screening of the catalysts, studies of the reaction mechanism, and solvent effects were investigated in detail as follows.

B. Screening of catalysts

As outlined in Fig. 1, a series of potential catalysts was examined for the oxa-Michael reaction between PEG and DVS. To quantify the catalytic activities with NMR measurements, triethylene glycol monomethyl ether (M-EG₃-OH, $n = 3$, $R = \text{CH}_3$) was employed as a model compound for PEG. The results are summarized in Table I. The catalyst-free control experiment showed little reaction between M-EG₃-OH and DVS. NEt₃, pyridine, and PDA produced hardly any conversion of the reactants toward the desired product in 2 h at room temperature, although amine was reported to be able to catalyze the reaction at high temperature.²⁸ DABCO, 1-MIM, and DMAP all led to the desired product with considerable conversion in 2 h. Among them, DMAP showed the highest catalytic activity, giving a 93.5% conversion. Some of the P-centered nucleophiles also promoted the oxa-Michael reaction,^{34,38} where TPPTS gave a conversion of 35.2% and PPh₃ gave a conversion of 91.5%. But TCEP showed the least catalytic activity because of its low solubility in the reactant mixture. Therefore, DMAP was selected as the catalyst of choice for further studies.

Using EG₆ ($n = 6$ and $R = \text{H}$) as a model compound of PEG, DMAP was employed to synthesize hydrogel precursors under solvent-free conditions. To minimize the potential crosslinking, excess DVS (8 eq.) was stirred with EG₆ at room temperature with catalytically equivalent DMAP. The reaction gave a conversion of 91% in 2 h, indicating the high

SCHEME 1. One-step synthesis of PEG-VS through the reaction between PEG and DVS enabled by organic nucleophiles.

Fig. 1. Oxa-Michael reaction of M-EG₃-OH with excess DVS catalyzed with various catalysts in the solvent-free system at room temperature.

catalytic activity of DMAP. This facile strategy significantly simplifies the synthesis of VS group terminated hydrogel precursors and would likely facilitate the studies related to PEG-VS.

C. Reaction mechanism

To explain the mechanism of the DMAP catalyzed oxa-Michael reaction, we proposed a nucleophile-mediated pathway, as illustrated in supplementary material Fig. 1.⁴³ First, DMAP attacks the double bond of DVS to form an ylid. Next, the ylid reacts with PEG, generating a cation intermediate and a deprotonated PEG molecule. The deprotonated PEG reacts with DVS to afford a carbanion intermediate. The PEG-VS product can then be afforded by protonation of the carbanion intermediate with a PEG molecule, and the deprotonated PEG participates in another catalytic cycle. It

is likely that the protonation process is the rate-limiting step and the carbanion structure is the most stable intermediate in this reaction. To substantiate the reaction mechanism, DVS reacted with deuterated methanol in the presence of DMAP, and ¹H NMR was employed to track the reaction. In the spectrum (supplementary material Fig. 2), a broad peak at around 4.60 ppm (peak 1) can be assigned to the negatively charged methylidyne in the carbanion intermediate. After protonation, the peak shifted to 3.75 ppm (peak 3). As expected, the total peak area of peaks 1 and 3 equals 1/2 of the peak at around 3.78 ppm (peak 2), which can be assigned to $-\text{SO}_2\text{CHDCH}_2$. The results suggested the formation of a stable carbanion intermediate during the reaction.

D. Solvent effect

The solvent effect on the DMAP catalyzed oxa-Michael reaction was next investigated to evaluate the applicability of this reaction on hydroxyl containing compounds with low solubility in DVS. The first solvent examined was water. It was observed that DVS reacted violently with water, resulting in mainly hydrolysis products of DVS. This result implied that the anhydrous condition is better suited for this DMAP catalyzed reaction. We then screened eight aprotic solvents, including acetone, ethyl acetate, dichloromethane, chloroform, acetonitrile (CH_3CN), tetrahydrofuran, dimethyl sulfoxide (DMSO), and N,N-dimethylformamide (DMF). As summarized in supplementary material Table I, all these solvents showed various degrees of adverse effects on the rate of the reaction, mostly giving less than 20% conversion in 12 h with the exception of CH_3CN , DMSO, and DMF. In the case of CH_3CN , a conversion of 71.0% was achieved. The inferior outcome in these solvents as compared to the aforementioned solvent-free condition could be due to the charged ylid intermediate being unstable in the less polar

TABLE I. Oxa-Michael reaction of M-EG₃-OH and DVS in the solvent-free system at room temperature with/without the catalyst.

Entry	M-EG ₃ -OH (eq.)	DVS (eq.)	Catalyst (0.1 eq.)	Conversion (%) ^a
1	1	4	No Cat.	— ^b
2	1	4	NEt ₃	— ^b
3	1	4	Pyridine	— ^b
4	1	4	PDA	— ^b
5	1	4	DABCO	7.4
6	1	4	1-MIM	31.5
7	1	4	DMAP	93.5
8	1	4	TPPTS	35.2
9	1	4	PPh ₃	91.5
10	1	4	TCEP	3.6 ^c

^aConversion of M-EG₃-OH was determined using ¹H NMR spectroscopy after 2 h of reaction.

^bProduct was not detected.

^cNEt₃ of 0.5 eq. was added to provide a basic condition.

FIG. 2. (a) Schematic of PEG gel preparation through the DMAP catalyzed oxa-Michael reaction. (b) Photographs of gels prepared through the DMAP catalyzed oxa-Michael reaction of EG₆ and DVS after 1 h of reaction.

aprotic solvents. It is worth noting that a conversion of 91.5% was obtained when the reaction in CH₃CN was prolonged to 24 h. By using CH₃CN, this reaction was successfully applied to ethylene glycol polymers with various molecular weights and structures (supplementary material Table II). DMF and DMSO also produced a considerable amount of vinyl sulfone terminated derivatives in 12 hours, indicating the potential of applying such a catalytic oxa-Michael reaction for the modification of bioactive carbohydrates (e.g., hyaluronic acid) in solvents.

E. PEG hydrogel formation

To demonstrate the feasibility of the DMAP catalyzed oxa-Michael reaction for PEG gel preparation, EG₆ and DVS reacted with a mole ratio of 1:1. Although hydrogel formation was not observed, a water-soluble linear polymer was obtained, which has a number average molecular weight (M_n) of 1350 g/mol and a polydispersity index of 1.42 (supplementary material Fig. 3). To achieve a stable network, polyhydroxy compounds should be included in the reaction system. Here, we selected glycerol, a tri-functional alcohol, as a crosslinker to form a network structure. Specifically, hexaethylene glycol (EG₆), glycerol, and DVS were mixed at room temperature, and gelation took place when DMAP was added [Fig. 2(a)]. As the ratio of glycerol to EG₆ increased, the reactant mixture showed a quicker loss of fluidity [Fig. 2(b)]. This decrease in the gelation time can be attributed to the quicker formation of the network structure.

The freshly prepared gels were washed with methanol to remove the unreacted molecules and fully hydrated in water to achieve PEG hydrogels. The crosslinking degree, water content, and residual vinyl sulfone group content of the PEG

hydrogels prepared with different ratios of glycerol to EG₆ are summarized in supplementary material Table III. The crosslinking degree of all the formed gels was greater than 92%, proving the high catalytic activity of DMAP. The water content of the hydrogels decreased as the ratio of glycerol to EG₆ increased. This is because a higher amount of crosslinker leads to greater crosslinking, forming a more rigid network structure. The rigid structure would absorb less water due to the low deformability. The residual vinyl sulfone group content of all the hydrogels is less than 1%, which is likely due to the fact

FIG. 3. Increase in the amount of glycerol in the reactant led to the increase in the storage modulus of the as-prepared gels and fully hydrated gels. The storage moduli of samples prepared with a 1:1 ratio of glycerol to EG₆ were not attainable because of the long gelation time (more than 24 h). An angular frequency of 1 Hz and a deformation amplitude $\gamma^0 = 0.01$ were used for all storage moduli measurements.

that ethanol used in the washing step is able to consume the unreacted vinyl sulfone group under the catalytic condition. Using aprotic solvents (e.g., CH₃CN) instead of ethanol, the unreacted vinyl sulfone groups could be preserved as active sites for further functionalization.

The storage moduli (G') of these gels and hydrogels were also measured, as shown in Fig. 3. Due to the long gelation time (more than 24 h), the storage moduli of samples prepared with a 1:1 ratio of glycerol to EG₆ were not attainable. The storage modulus of the fully hydrated gel is unusually higher than that of the as-prepared gels.³⁹ This might be because the hydrogel formed in nonaqueous conditions has a less porous structure, and the hydration process formed plenty of hydrogen bonds without significantly softening the networks. The storage modulus of the fully hydrated hydrogels is extraordinarily higher than that of normal PEG hydrogels,^{9,13} likely due to the extraordinarily greater crosslinking sites in the hydrogels formed with oligomeric ethylene glycol. As the amount of glycerol increased, a higher G' of the gels and hydrogels was observed, indicating the increase in the resilience of the network. When 100% glycerol was employed, the mixture instantly formed a rigid gel, and the G' value was difficult to measure. These results demonstrated that the mechanical properties of the PEG hydrogels can be easily controlled by the amount of crosslinker introduced. The mechanical properties were also influenced by the chain length of the PEGs. The increase in the PEG chain length resulted in an increase in G' of the hydrogel as well (supplementary material Fig. 4).

F. Protein adsorption

To evaluate the ability of PEG hydrogels prepared with different ratios of glycerol to EG₆ to resist nonspecific protein adsorption, FITC-BSA was used as a model protein to assess protein adsorption, as shown in Fig. 4. All the hydrogels show lower FITC-BSA adsorption (less than 70 μg of protein per gram hydrogel) than most of the hybrid PEG

hydrogels,^{40,41} indicating good antinonspecific protein adsorption ability. A statistical difference in protein adsorption was observed between hydrogel samples prepared with different ratios of glycerol to EG₆ ($p < 0.05$ in the pairwise comparisons, $n = 4$). The difference in the ability to resist nonspecific protein adsorption can be attributed to the differences in the amount of crosslinking points in the gel network. The hydrogels prepared with a higher ratio of glycerol to EG₆ have more crosslinking points, forming a more compact and hydrophobic structure, which could cause more nonspecific protein adsorption. On the other hand, the hydrogels prepared with a lower ratio of glycerol to EG₆ have less crosslinking points, possibly forming a hydrophilic and loose structure with a greater surface area for protein adsorption. Among these samples, the hydrogel prepared with a 3:1 ratio of glycerol to EG₆ has the best antinonspecific protein adsorption ability, with 11 $\mu\text{g}/\text{g}$ of FITC-BSA adsorption in 2 h and 17 $\mu\text{g}/\text{g}$ of adsorption in 24 h.

G. In vitro cytotoxicity of PEG hydrogels

The cytotoxicity of the PEG hydrogels was assessed using direct contact assay with bone marrow stromal cells. The viability of the cells was quantified using the MTT test and is illustrated in Fig. 5, where none of the PEG hydrogel treatments resulted in significant cell death as compared to the negative control ($p > 0.05$ in pairwise comparisons with the control). As comparison, the positive control showed less than 5% of the cell viability. The cytotoxicity assessment with 3T3 mouse fibroblast cells showed the same result, where no obvious morphological change was observed in cells treated with PEG hydrogels for 24 h (supplementary material Fig. 5). These results indicate the low cytotoxicity of the PEG hydrogels, which is consistent with the previous report.⁴²

H. Drug release

Using Nile Red as a model drug, the applicability of the PEG hydrogels for controlled drug release was studied. Drug

FIG. 4. FITC-BSA adsorption on the PEG hydrogels prepared with different ratios of glycerol to EG₆.

FIG. 5. Viability of the cells with PEG hydrogel treatment normalized to the untreated control group using the MTT test.

FIG. 6. Cumulative release of Nile Red (a) and oleanic acid (b) from hydrogels prepared with different ratios of glycerol to EG₆.

encapsulation was realized by immersing the hydrogels in the drug solutions for 12 h, and the encapsulation efficiency for all the hydrogels was calculated to be higher than 60%. Then, the hydrogel was incubated in water at 37 °C to study the drug release performance, and the results are illustrated in Fig. 6(a). All the hydrogels exhibited a burst release of around 25% Nile Red on the first day, followed by a slow release. As expected, the hydrogels prepared with a higher glycerol ratio to EG₆ showed slower drug release. This is because the hydrogels with higher glycerol contents have a more hydrophobic and rigid structure, and the hydrophobic effect could slow down the release of hydrophobic Nile Red.

Then, the PEG hydrogels were applied in the controlled release of oleanic acid, which is a relatively nontoxic hepatoprotective drug. The encapsulation efficiency for all the hydrogels was calculated to be higher than 50% after immersing the hydrogels in the drug solution for 12 h. A drug release curve similar to Nile Red was obtained when incubating the drug-loaded hydrogels in water [Fig. 6(b)]. A faster release of oleanic acid was observed compared to Nile Red, which can be attributed to oleanic acid being less hydrophobic. Since the glycerol to PEG ratios can be adjusted in a wide range, the drug release rate can thus be controlled accordingly.

IV. SUMMARY AND CONCLUSIONS

In conclusion, DMAP could efficiently catalyze the oxa-Michael reaction between PEGs and DVS, which enables the one-step synthesis of the VS group terminated hydrogel precursor under mild conditions. This reaction significantly simplifies the preparation of PEG-VS, facilitating the studies of PEG hydrogels related to PEG-VS. Polar aprotic solvent provides the feasibility of synthesizing vinyl sulfone derivatives of other hydroxyl-bearing compounds, expanding the range of substrates to prepare hydrogels for versatile applications.

Furthermore, the DMAP catalyzed oxa-Michael reaction was applied for the facile preparation of PEG hydrogels by

adding glycerol as a crosslinker. The mechanical properties of the gels are tunable simply by varying the crosslinker concentration and the PEG chain length. These hydrogels are antifouling and exhibit no significant cytotoxicity toward bone marrow stromal cells and 3T3 fibroblast cells. Potential drug delivery application was demonstrated with the hydrophobic compound of Nile Red and oleanic acid, where the release rate was easily controlled by the crosslinking density of the hydrogels. The synthetic strategy for hydrogel preparation reported herein can be implemented to other types of hydroxyl group bearing compounds, such as PVA and polysaccharides, thus greatly diversifying the library of hydrogels for biomedical applications.

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