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# Injectable drug-loaded hydrogel using "clickable" amphiphilic triblock copolymer as precursor†

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**We developed a facile strategy to prepare injectable drug-loaded hydrogel with chemical crosslinkages. PCL-POEGM-PCL amphiphilic triblock copolymer was synthesized in "one pot" by a combination of polycondensation and ring-opening polymerization, which can disperse hydrophobic drug in aqueous solution, and be crosslinked by POEGMS under physiological conditions.**

Hydrogels are three-dimensional, cross-linked networks of water-soluble polymers[1, 2] and permit loading of drugs into the gel matrix and subsequent drug release at a rate dependent on the diffusion coefficient of the small molecule or macromolecule through the gel network.[3, 4] Recently this distinguishing feature has sparked particular interest in their use in drug delivery applications.[5, 6] Compared to other polymeric systems (micelles, microspheres and micro/nanogels) for drug delivery administrated by intravascular injections, hydrogels loaded with drugs can be used for local treatment and have the advantages to overcome challenges that premature release in the circulatory system transports drugs throughout the body and discounted local drug concentration.[7-9]

In contrast to surgical implant systems, injectable hydrogels may be easily realized through a simple, minimally invasive injection of their aqueous solutions.[10] Thermosensitive amphiphilic polymers exhibit sol-gel phase transition in response to changes near physiological temperature, and can be used as injectable hydrogels.[11, 12] However, the deficiency of chemical crosslinking points may limit their applications. Most recently, injectable hydrogels formed via "clickable" precursors have received intensive research because of the emerging of "click" chemistry.[13] The first "click" reaction defined by Sharpless[14] is azide/acetylene cycloaddition reaction catalyzed by Cu(I), and has been an approach to prepare hydrogels.[15, 16] However, the technique is unsatisfied with requirements of ideal injectable hydrogels because of bio-toxic metal catalyst. Subsequently, mild and biocompatible "click" reactions, such as hydrazone reactions,[17] Schiff base reaction,[18, 19] thiol-ene/yne reaction[20, 21] and oxime reaction,[22] are redefined and utilized to *in situ* form hydrogels by biomaterials researchers.

Poly(ethylene glycol)s (PEG) now are the golden materials in biomedical applications because they are hydrophilic, nontoxic, absent of antigenicity and immunogenicity, and can be directly excreted by the kidneys. Based on the above mentioned features, PEG has been approved by Food and Drug Administration (FDA), and no other synthetic polymer has yet reached the status of PEG in the field of biomedicine.[23-26] Using clickable PEG derivatives to prepare injectable hydrogels has been an interesting topic. For example, Robert Langer's group has prepared thiol-ene hydrogels based on tri-thiol-functionalized ethoxylated-polyol esters (TEPEs) and PEG diacrylate (PEGDA) for the delivery and release of methylprednisolone sodium succinate.[27] In our previous work, we have developed a facile method to synthesize biodegradable multifunctional PEG derivatives by the polycondensation of oligo(ethylene glycol) diol (OEG) with functional diacids, such as mercaptosuccinic acid,[28, 29] maleic acid[29] and malic acid.[30, 31] Injectable hydrogels with chemical crosslinkages could be prepared through thiol-ene "click" reaction by simply mixing the aqueous solution of the PEG derivatives containing multiple thiols and enes under physiological conditions.[29, 32, 33]

However, loading hydrophobic drugs into PEG-based hydrogels homogenously remains challenges because the framework of hydrogels is hydrophilic. Typically, the above mentioned thermal-induced hydrogels are formed by PEG based amphiphilic triblock copolymers with hydrophobic blocks, such as poly(lactic acid) (PLA),[34] poly(lactic acid-*co*-glycolic acid) (PLGA),[35] poly(ε-caprolactone) (PCL),[36] which can

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† Electronic Supplementary Information (ESI) available: [experiment details, GPC trace and $^1$H NMR spectrum of POEGMS, gelation time, FT-IR spectra, SEM images, swelling ratio and rheology measurements]. See DOI: 10.1039/x0xx00000x.

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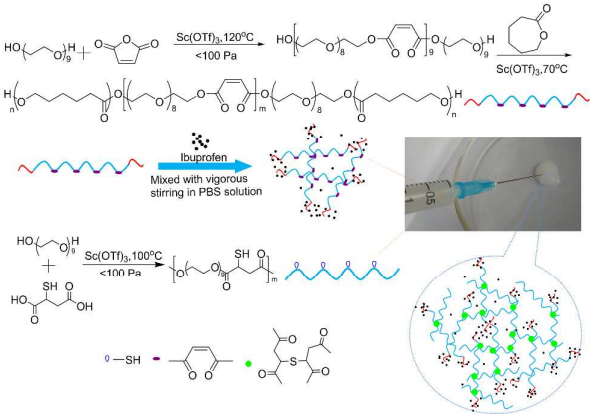
efficiently disperse hydrophobic drug in aqueous medium. Based on these reports and our previous work,[37] we developed a facile strategy to synthesize amphiphilic triblock copolymer containing multiple "clickable" groups, which was used as injectable hydrogel precursor to load hydrophobic drugs, and chemically crosslinked via thiol-ene "click" reaction under physiological conditions. First, polycondensation of 10 molar equivalent OEG with 9 molar equivalent maleic acid (MA) at 120 °C utilizing scandium trifluoromethanesulfonate [$Sc(OTf)_3$] as catalyst was conducted to synthesize unsaturated poly[oligo(ethylene glycol) maleate] (POEGM) terminated with two hydroxyl groups. Sequentially, the polymerization system was cooled to 70 °C, followed by the injection of ε-caprolactone (CL) under nitrogen atmosphere. The controlled ring-opening polymerization (ROP) of CL was initiated by the end hydroxyl groups of POEGM in the presence of the $Sc(OTf)_3$, resulting in poly(ε-caprolactone)-*b*-poly[oligo(ethylene glycol) maleate]-*b*-poly(ε-caprolactone) (PCL-POEGM-PCL) amphiphilic triblock copolymers. Water soluble poly[oligo(ethylene glycol) mercaptosuccinate] (POEGMS) ($^1$H NMR spectrum and GPC trace of POEGMS are shown in Fig. S1 and S2) with multiple thiols was synthesized according to our previous reports[29], which was used as another precursor to form hydrogel with drug loaded PCL-POEGM-PCL aqueous solution. The full synthetic routes and preparation of drug-loaded hydrogel are exhibited in Scheme 1.




Scheme 1 Preparation of hydrophobic drug loaded injectable hydrogel with chemical crosslinkages.

The $^1$H NMR spectrum of POEGM (Fig. 1A) clearly demonstrates the formation of ester bonds ($H^b$) and the retention of double bond ($H^a$) in the polymer. The typical $^1$H NMR spectrum of PCL-POEGM-PCL is shown in Fig. 1B. Peaks at 1.4 ($H^f$), 1.6 ($H^e$), 2.3 ($H^d$) and 4.1 ($H^g$) ppm are assigned to the signals of PCL block. The molecular weight, molecular weight distribution and chemical composition of POEGM and PCL-POEGM-PCLs, as well as those of POEGMS, are summarized in Table 1. From the GPC traces (Fig. 1C) of POEGM, PCL-POEGM-PCL1 and PCL-POEGM-PCL2, we believe that the chain growth polymerization of CL was initiated by the end hydroxyl groups of POEGM, and no PCL homopolymer was obtained. The chain length of PCL block could be well controlled by the feed molar ratio of CL to OEG.

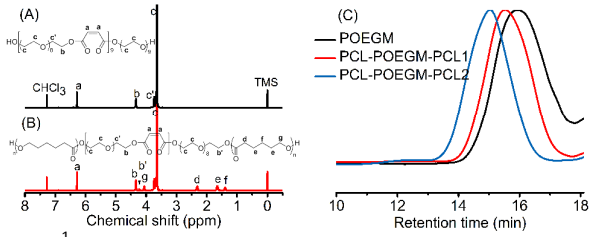



**Fig. 1** $^1$H NMR spectra of (A) POEGM and (B) OCL-POEGM-OCL1; (C) GPC traces of POEGM, PCL-POEGM-PCL1, PCL-POEGM-PCL2.

**Table 1** Polymers synthesized in this study.

| Polymer | $M_n$ [a] | PDI [a] | OEG: CL [b] (in feed) | OEG: CL [c] (in polymer) |
|---|---|---|---|---|
| POEGM | 3,700 | 1.80 | – | – |
| PCL-POEGM-PCL1 | 6,100 | 1.73 | 1: 1.2 | 1: 1.0 |
| PCL-POEGM-PCL2 | 12,800 | 1.66 | 1: 2.4 | 1: 2.2 |
| POEGMS | 9,100 | 1.97 | – | – |

[a] Measured by GPC; [b] molar ratio; [c] molar ratio, determined by $^1$H NMR in $CDCl_3$ based on ethylene glycol (EG) unit ($H^{c, c'}$, 3.6, 3.7 ppm) and CL units ($H^{d, e, f}$, 2.3, 1.6 and 1.4 ppm).

The water solubility of the synthesized polymers was investigated, and ibuprofen was used as a model hydrophobic drug to be dispersed into the aqueous solution of the polymers. As shown in Fig. 2, PCL-POEGM-PCL2 is insoluble in water due to its relatively long hydrophobic PCL block. POEGM, POEGMS and PCL-POEGM-PCL1 are all soluble in water; however, only the aqueous solution of PCL-POEGM-PCL1 can disperse ibuprofen homogeneously, which may be driven by the hydrophobic interactions between ibuprofen molecules and PCL blocks. The solution of PCL-POEGM-PCL1 with dispersed ibuprofen could *in situ* form hydrogel with POEGMS through thiol-ene "click" reaction under physiological conditions. Gelation time was measured by test-tube inversion method and the results were shown in Fig. S3. Thiol-ene reaction is fast and 3D hydrogels network was formed rapidly within one or two minutes varied by the solid contents, which makes it a suitable candidate as an injectable implant biomaterial. FT-IR analysis further confirmed the high efficiency of "click" reaction (Fig. S4). The absorptions of the C=C stretch at 1636 $cm^{-1}$ and the S–H stretch at 2538 $cm^{-1}$ almost disappeared after gelation, which indicates the high conversion of the thiol–ene "click" reaction and the perfect 3D network of the obtained hydrogel.

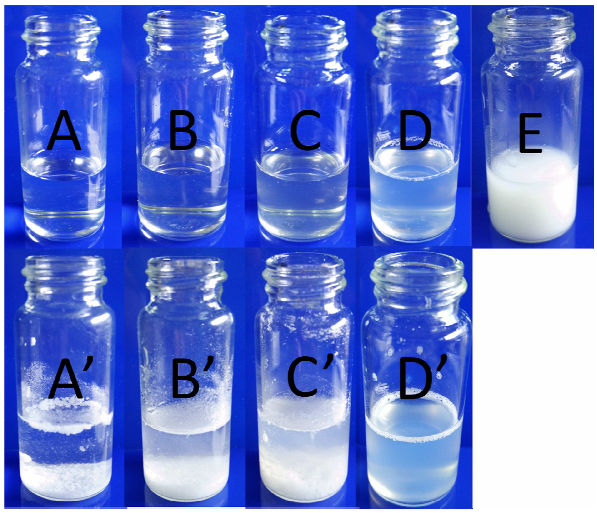



**Fig. 2** Pictures of water solubility of polymers and the solubility of ibuprofen in polymer aqueous solutions: (A) deionized water without polymer; (B) POEGMS in deionized water; (C) POEGM in deionized water; (D) PCL-POEGM-PCL1 in deionized water; (E) PCL-POEGM-PCL2 in deionized water; (A') ibuprofen in deionized water; (B') ibuprofen in aqueous solution of POEGMS; (C') ibuprofen in aqueous solution of POEGM; (D') ibuprofen in aqueous solution of PCL-POEGM-PCL1. The concentration of the polymer solutions is 30 wt%, the weight fraction of ibuprofen to polymer is 10 %.

The state of ibuprofen in the hydrogel was analysed by differential scanning calorimeter (DSC) and X-ray diffractomer (XRD). As can be seen in Fig. 3A, the melting temperature of pure ibuprofen is 78 °C. However, no melting peak was detected from dry ibuprofen loaded triblock copolymer and hydrogel. It can thus be concluded that the ibuprofen in hydrogel is amorphous or molecularly dispersed. The information obtained by XRD (Fig. 3B) agree with the result from DSC analysis. The disappearance of the diffraction peak of ibuprofen in dry hydrogel suggests that ibuprofen in hydrogels is in an amorphous state. Based on the above investigation and analysis, we believe that it is the PCL blocks that dispersed the hydrophobic and crystalline ibuprofen.

The morphology, moduli, swelling ratio and degradation of resultant ibuprofen loaded hydrogels with 10, 20 and 30 wt% concentration of precursors were investigated. The existence of porous structure is indispensable to allow for diffusion of drugs. As seen in Fig. S5, scanning electron microscopy (SEM) images of the lyophilized hydrogel clearly demonstrate the presence of a porous structure. The percentage of pore fractions of hydrogel with 10 wt% solid content is highest and that of hydrogel with 30 wt% solid content is lowest, which is due to the increase of crosslinking densities with the high solid content. The moduli of hydrogels also increased with the increasing concentration of precursors as shown in Fig. S6. The storage modulus (G') of hydrogel with 10 wt% solid content is 1.1 kPa, and the G' increased up to 47 kPa with a solid content of 30 wt%, due to the dense network with high concentration of precursors. For the same reason, swelling ratio of hydrogels with 30 wt% concentration of precursors is lower than that of other hydrogels (Fig. S7). All of the hydrogel reached equilibrium swelling within 12 h. The obtained hydrogels can be fully degraded. As exhibited in Fig. 3C, the full degradation time of hydrogel with a solid content of 30 wt% is about 23 days, which is slightly longer than that of hydrogel with low solid content (10 wt%, about 21 days).

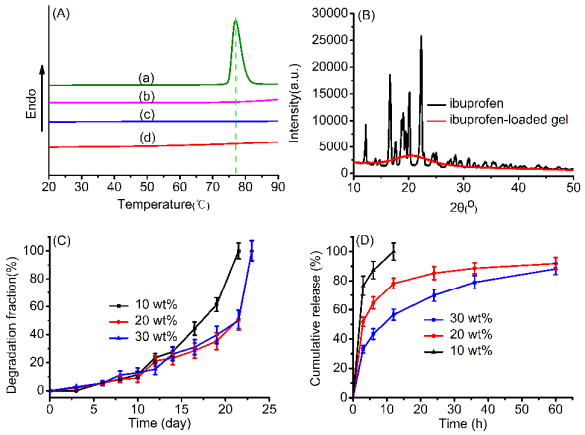



**Fig. 3** (A) DSC curves of native ibuprofen (a), PCL-POEGM-PCL1 (b), freeze-dried PCL-POEGM-PCL1 loaded with ibuprofen (c) and freeze-dried hydrogel loaded with ibuprofen (d); (B) XRD spectra of the native ibuprofen and freeze-dried ibuprofen-loaded hydrogel; (C) degradation profiles of hydrogels with various solid contents; (D) ibuprofen release profile from hydrogels with various solid contents.

The *in vitro* ibuprofen release was carried out in pH 7.4 PBS solution at 37 °C. The drug loading content is 10 wt% to the precursors. Fig. 3D shows the cumulative release percentage of ibuprofen from hydrogels with 10, 20 and 30 wt% solid content. A burst ibuprofen release can be observed from the hydrogel with a solid content of 10 wt%. About 80 % of loaded ibuprofen was released within 2 hours, and almost all ibuprofen was released after 12 hours. However, sustained release behaviors can be observed from the hydrogels with high solid contents. For hydrogel with a solid content of 30 wt%, the time for 80 % ibuprofen release could be prolonged to 60 hours. High solid content results in dense hydrogels network, which impedes diffusion of ibuprofen from hydrogel matrix to release medium; meanwhile, hydrophobic interaction between ibuprofen and PCL blocks also retards the release of ibuprofen. This kind of injectable hydrogels loaded with ibuprofen may be used as a local delivery system for arthritis treatment.[38]

Cell viabilities of precursors and hydrogels were evaluated by Cell Counting Kit-8 (CCK-8) assay. Hela cells were incubated in various concentrations of PCL-POEGM-PCL1 or POEGMS for 24 h. As shown in Fig. 4, no significant cytotoxicity on the growth inhibition of Hela cells can be observed even when the concentration of POEGMS and PCL-POEGM-PCL is very high (10 mg/mL). Meanwhile, the obtained hydrogels also show low cytotoxicity to Hela cells, even when the solid content is up to

30 wt%. Therefore, the present hydrogels have significant potential as biomaterials.

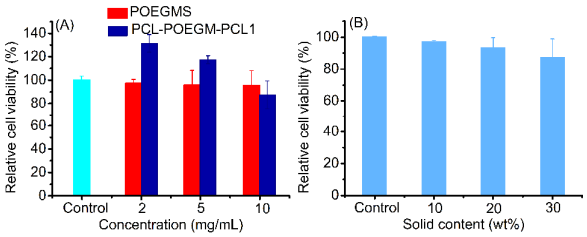



**Fig. 4** Relative cell viability of (A) precursors and (B) hydrogels to Hela cells.

In conclusion, a facile method was presented to synthesize amphiphilic triblock copolymer with multiple "clickable" groups, which can efficiently disperse hydrophobic drug in aqueous solution, and undergo thiol-ene "click" reaction under physiological conditions to give drug-loaded injectable hydrogels. This kind of hydrogels show low cytotoxicity to Hela cells, and can sustainedly release loaded drugs during more than 60 hours, which makes it a potential candidate as drug carrier for localized and sustained delivery of therapeutic drugs.

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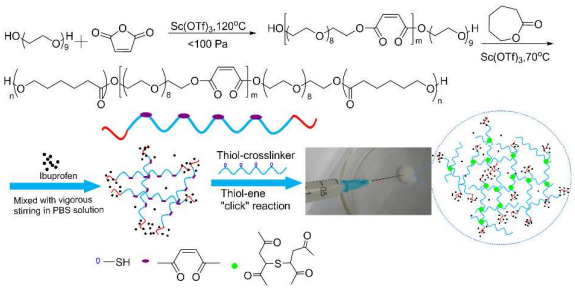
## Graphic abstract




Poly(ε-caprolactone-*b*-poly[oligo(ethylene glycol) maleate]-*b*-poly(ε-caprolactone) (PCL-POEGM-PCL) amphiphilic triblock copolymer was facilely synthesized in "one pot" by a combination of polycondensation and ring-opening polymerization, which can disperse hydrophobic drug in aqueous solution, and be crosslinked by poly[oligo(ethylene glycol) mercaptosuccinate] (POEGMS) under physiological conditions.