

## Research Article

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# Synthesis of PEGylated polypeptides bearing thioether pendants for injectable ROS-responsive hydrogels

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**Abstract:** Injectable hydrogels have gained massive attention in the past decade due to the simple operation, the ability to fill irregularly shaped wounds, and high safety. Herein, we design a PEGylated polypeptide with reactive oxygen species (ROS) responsiveness for injectable hydrogel. In brief, poly(ethylene glycol)-block-poly(2-methylthioethyl L-glutamate) was synthesized by ring opening polymerization of *N*-carboxylic anhydride with thioether group. The obtained amphiphilic copolymer could self-assemble into micelles, which underwent a sol–gel phase transition at 35°C. Carbon-13 nuclear magnetic resonance, dynamic light scattering, and circular dichroism measurements indicated that the partial dehydration of poly(ethylene glycol), the aggregation of micelles, and the change of  $\beta$ -sheet packing of PMLG<sub>15</sub> as temperature increasing were regarded as the main driving forces of gelation. The formed hydrogel was disrupted by 10 mM H<sub>2</sub>O<sub>2</sub> due to the oxidation of hydrophobic thioether groups into hydrophilic sulfoxides. Furthermore, *in vitro* experiment showed that the hydrogels consisting of thioether groups exhibited cytoprotective ability against the damage of H<sub>2</sub>O<sub>2</sub>-induced oxidative stress to L929 cells. Finally, the aqueous solution of this copolymer rapidly

transformed into gel after being injected into the subcutaneous layer of mice.

**Keywords:** polypeptides, injectable hydrogels, oxidative sensitivity, block copolymers, thioether

## 1 Introduction

Polymeric hydrogels with an obvious three-dimensional network structure and abundant water content have been widely used in biomedical fields, due to their similarity in both composition and mechanical properties to the soft tissue in the body (1,2). Among them, injectable physically cross-linked hydrogels have attracted increasing attention because of the unique sol–gel phase transition characteristic, the ability to fill irregularly shaped wounds, and adhesion to wound tissue (3,4). In addition, injectable hydrogels offer great advantages for localized drug delivery (5,6). At room temperature, the aqueous solution of the polymer has good fluidity and can evenly mix the active substances such as anticancer drugs, proteins, peptides, and nucleic acids. After injection, the polymer solution rapidly transits to a gel state at the temperature around 37°C, and the loaded drugs are released in a controllable way to achieve long-term therapeutic effect. This type of treatment maximizes the biological activity of the active substance (7,8).

Due to the aforementioned features, various biopolymer-based injectable hydrogels have been developed for biomedical applications. Wei's group designed an injectable, thermosensitive chitosan hydrogel as a sustained-release platform for two-dimensional peptide nanosheet–doxorubicin (DOX) conjugates. This novel delivery system not only increased the solubility and bioavailability of DOX, but also improved the internalization of the hydrophobic DOX into tumor cells, achieving an enhanced cancer cell-killing efficacy (9). A temperature-sensitive injectable hydrogel has been developed based on the poly(D,L-lactide)-poly(ethylene glycol)–poly(D,L-lactide) micelles. The controlled drug release was achieved by compounding the hollow

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mesoporous silica containing erlotinib into the gel system (10). Ding's group designed and synthesized a series of diblock or triblock copolymers of poly(ethylene glycol) (PEG) and poly(D,L-lactide-co-glycolide), and used them to prepare injectable hydrogels (11). They found that thermogelation is a reversible physical process primarily driven by the self-assembly of amphiphilic block copolymers in aqueous solutions into micelles. As the temperature increases, the hydrophilic blocks (such as PEG) thermally contract, leading to the exposure of the hydrophobic core, forming semi-bald micelles. With further temperature rise, hydrophobic interactions are enhanced, promoting the aggregation of micelles into a percolated network. The hydrophobic channels within this network act as physical cross-linking points, eventually resulting in the formation of a gel network (12). Hydrogels have great potential in biomedicine and can be used as carriers of NO (13), paclitaxel (14), and other drugs as well as liquid embolization agents (15). However, those polyester/polyether materials are hard to be functionalized and show obvious acid microenvironment after degradation.

Reactive oxygen species (ROS)-responsive injectable hydrogels are of particular interest owing to its relevance to anti-inflammatory and anticancer treatments. Wang *et al.* synthesized a ROS-sensitive cross-linked polyvinyl alcohol (PVA) hydrogel to provide basic fibroblast growth factor for myocardial repair (16). Qiu *et al.* developed ROS-responsive hydrogel by mixing phenylboronic acid-grafted hyaluronic acid and PVA, and the classical iron chelator desferoxamine was loaded into the hydrogel to treat traumatic brain injury by eliminating iron deposition and scavenging ROS (17). In another study, phenylboronic acid-modified gelatin methacryloyl and epigallocatechin-3-gallate (EGCG) rapidly formed hydrogel and release anti-inflammatory agent EGCG in ROS and acidic conditions for ameliorating intervertebral disk degeneration (18). Although phenylboronate bond or boronic ester bond is sensitive to ROS, a second component with cis-dihydroxy groups is always needed in these hydrogels forming via this dynamic covalent bonding, leading to a more complicated gel preparation process (19). It is urgent to develop biomaterials that can form ROS-responsive and injectable hydrogels with a single component.

Polypeptide or polyamino acids are excellent biomedical materials due to the biodegradability, biocompatibility, regular secondary structures, and the easy functionality (20,21). Stimuli-responsive peptide assemblies and the biomedical applications have been summarized elsewhere (22,23). Our group has designed ROS-responsive PEGylated polypeptide bearing thioether pendants for anticancer drug delivery (24), and synthesized tetra-poly(ethylene glycol)-*b*-oligo (L-methionine) copolymers that can form an injectable and oxidation-sensitive hydrogel via the hydrophobic

interactions between the side chains of L-methionine (25). In this study, a ROS-responsive polypeptide block copolymer bearing thioether pendants, poly(ethylene glycol)-*block*-poly (2-methylthioethyl L-glutamate) (mPEG<sub>2k</sub>-*b*-PMLG), was synthesized for the formation of injectable hydrogels. The chemical structures of the copolymers were characterized by hydrogen-1 nuclear magnetic resonance (<sup>1</sup>H NMR) spectroscopy. The mechanism of thermal-induced gelation was studied by employing carbon-13 nuclear magnetic resonance (<sup>13</sup>C NMR), dynamic light scattering (DLS), and circular dichroism (CD) test. The oxidation of the pendant thioether groups was checked by infrared spectroscopy. Methyl thiazolyl tetrazolium (MTT) assay was conducted to prove that the mPEG<sub>2k</sub>-*b*-PMLG hydrogels could protect normal cells from oxidative damage. Finally, the *in vivo* gelation was verified by subcutaneous injection of polymer solution to rats. The experimental results indicated that the degree of polymerization (DP) of the block copolymers was one of the most critical factors for the hydrogel forming. Copolymer mPEG-*b*-PMLG with too long hydrophilic PEG chains or too short hydrophobic PMLG segment showed quite different self-assemble behaviors and might not form hydrogels in water. Specifically, the obtained mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> hydrogels are temperature and oxidation dual-responsive porous materials with good biocompatibility and hold great potential for drug delivery. This work exhibited the accuracy and the beauty of polymer science.

## 2 Materials and methods

### 2.1 Materials and characterization

The starting material, 1-tert-butyl *N*-(tert-butoxycarbonyl)-L-glutamate (Boc-Glu-OTBu), was supplied by Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). 2-(Methylthio) ethanol was acquired from Energy Chemical (Shanghai, China). Methoxy (polyethylene glycol) (mPEG<sub>2k</sub>,  $M_n = 2,000$ ) was purchased from Ponsure Biotechnology Co., Ltd (Shanghai, China), and mPEG<sub>2k</sub>-NH<sub>2</sub> was synthesized following our previous procedure. Sodium hydroxide and tetrahydrofuran were purchased from Sinopharm Co., Ltd., and *N,N*-dimethylformamide (DMF) was obtained from Shanghai Qiangshun Chemical Reagent Co., Ltd. Dulbecco's modified eagle medium (DMEM) cell culture medium was purchased from Biotechnology Co., Ltd. Trypsin was purchased from Wuhan Seville Biotechnology Co., Ltd., and penicillin-streptomycin solution was purchased from Beijing Sevin Innovation Biology Co., Ltd. MTT was purchased from Shanghai Biyuntian Biotechnology Co., Ltd. Dichloromethane, sodium chloride, trimethylamine, ethyl acetate, anhydrous

magnesium sulfate, sodium bicarbonate, and other chemicals were all bought from Xilong Scientific Co., Ltd. (Guangzhou, China).

## 2.2 Synthesis of mPEG<sub>2k</sub>-*b*-PMLG

The monomer, *N*-carboxyanhydride from methylthioethyl L-glutamate (MLG-NCA), was synthesized following our previous protocols (24). The di-block copolymer was synthesized through the ring-opening polymerization of MLG-NCA with mPEG<sub>2k</sub>-NH<sub>2</sub> serving as a macroinitiator (Figure S1). Briefly, MLG-NCA and mPEG<sub>2k</sub>-NH<sub>2</sub> macroinitiator (molar ratio 17:1) were added in a round-bottom flask under nitrogen atmosphere and dissolved by dry DMF. Following a 72 h reaction period at 25°C, the solution was subsequently subjected to dialysis and lyophilization. The product mPEG<sub>2k</sub>-*b*-PMLG was obtained as white solid.

## 2.3 Preparation of mPEG<sub>2k</sub>-*b*-PMLG hydrogels

The mPEG<sub>2k</sub>-*b*-PMLG was dissolved in a 0.01 M PBS with a pH of 7.4 at 4°C. Different concentrations of polymer solutions were prepared. Subsequently, 0.5 mL of each polymer solution was transferred to a small glass vial and placed in a water bath with a programmed warming process of 1°C per minute. And each temperature was maintained for 5 min. At a specific temperature, the glass vial was inverted. If the polymer solution did not flow within 30 s, it was considered the hydrogels have formed. Each sample was subjected to this test in triplicate for accuracy.

## 2.4 Dynamic rheological analysis

Dynamic rheological analysis was conducted using a US 302 (Anton Paar rheometer) with 25 mm diameter plates, operated in oscillation mode, and a gap distance of 0.5 mm. For testing, a 300 µL polymer solution was placed on the sample table, and the edges of the sample were sealed with silicone oil to prevent moisture evaporation. The changes of energy storage modulus (*G'*) with temperature were recorded with testing parameters of a strain ( $\lambda$ ) of 1% and a frequency ( $\omega$ ) of 1 Hz. The heating rate was 0.5°C·min<sup>-1</sup>, and the temperature range of the analysis was from 20°C to 60°C.

## 2.5 Temperature sensitivity of the polymers

The polymer was dissolved in deionized water at a concentration of 0.05 mg·mL<sup>-1</sup>. The size and size distribution of the

self-assembled particles were measured by DLS at 20°C and 60°C, respectively. CD measurement was performed over a temperature range of 10°C–60°C to monitor the secondary structure of the polypeptide mPEG<sub>2k</sub>-*b*-PMLG. Prior to testing, the samples were equilibrated at each temperature for 10 min. <sup>13</sup>C NMR was performed in the temperature range of 20°C–50°C to assess the polymer structure.

## 2.6 Oxidative sensitivity of the polymers

The mPEG<sub>2k</sub>-*b*-PMLG was dissolved in 0.01 M PBS with 12.0 wt% concentration, and hydrogel was formed at 35°C. Then, the hydrogel was incubated with PBS solution containing 10 mM or 100 mM H<sub>2</sub>O<sub>2</sub>. After 6 h or 12 h, the degradation medium was removed by dialysis, and the buck products after degradation were collected, freeze-dried, and submitted for <sup>1</sup>H NMR and Fourier transform infrared spectroscopy test.

## 2.7 Cytotoxicity evaluation

The *in vitro* cytotoxicity was assessed by using MTT assay. First, L929 cells were cultivated in 96-well plates with a seeding density of 8,000 cells per well with 200 µL DMEM medium and incubated at 37°C with 5% CO<sub>2</sub> for 24 h. Afterward, the culture medium was removed, and 200 µL of oxidized polymer solution by H<sub>2</sub>O<sub>2</sub> or raw polymer solution in DMEM was added, with concentrations ranging from 0.063 to 2 mg·mL<sup>-1</sup>. It should be noted that the raw mPEG<sub>2k</sub>-*b*-PMLG solution formed hydrogel at 37°C. The control group was cultured with DMEM without polymer. Following an additional 24 h incubation, 20 µL MTT solution was added and incubated for 4 h. Then, the culture medium was discarded, and 150 µL of DMSO was added. The absorbance or optical density (OD) at 490 nm for each well was measured using a microplate reader. The cell viability rate (cell viability) was calculated using the following formula:

$$\text{Cell viability (\%)} = (A_{\text{sample}}/A_{\text{control}}) \times 100\%$$

## 2.8 Cellular protective effect of gels in oxidative environment

The L929 cells were cultured (8,000 cells/well, 200 µL DMEM/well) at 37°C for 24 h. The culture medium was removed, and 50 µL polymer solution (12 wt%) was added to each well to form a stable hydrogel on the cell surface. Subsequently, 180 µL of DMEM medium containing H<sub>2</sub>O<sub>2</sub> (0.125, 0.25, 0.5

or 1 mM) was added. After 24 h, the medium and gel were removed. Cells were washed with PBS for 3 times to remove the remaining small amount of gel. Then, 150  $\mu$ L DMEM culture solution was added. The cells that did not contain the gel were treated as the control group in the same protocols. MTT assay was used to detect the cell viability.

## 2.9 In vivo test

Female Sprague-Dawley (SD) rats (~200 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. All animal experimental protocols were approved by the Animal Care and Use Committee of Changchun Institute of Applied Chemistry (No. 2022-0082). The mPEG<sub>2k</sub>-*b*-PMLG solution (12.0 wt% in PBS) was subcutaneously injected into the back of SD rats through a syringe. The rats were killed 15 min later. The gel formation was checked, and photographs were taken.

# 3 Results and discussion

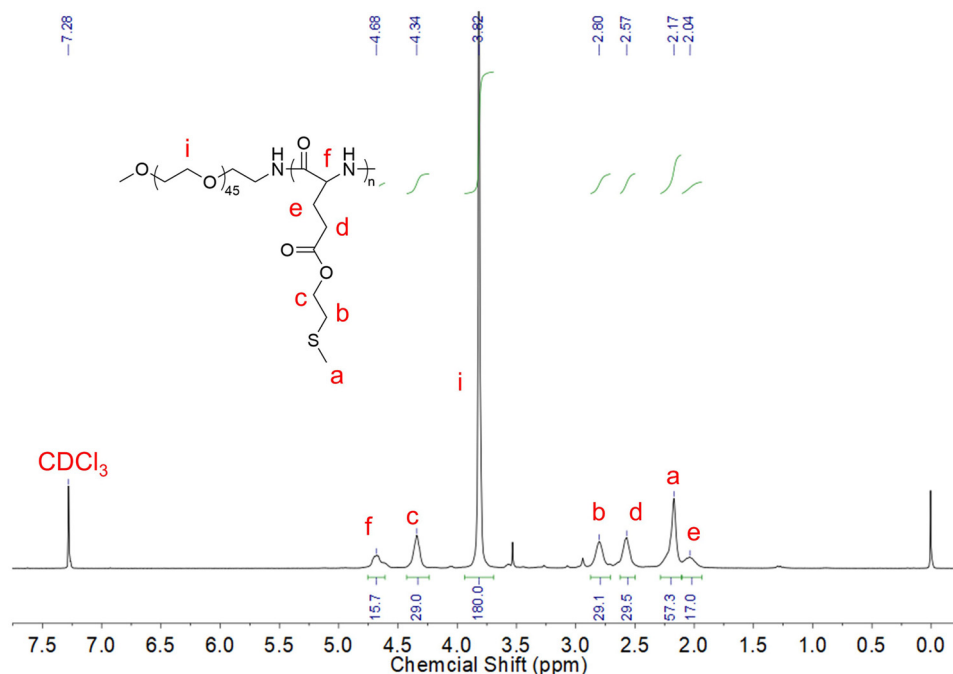
## 3.1 Characterization of mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub>

The synthesis pathway for mPEG<sub>2k</sub>-*b*-PMLG, which is sensitive to both oxidation and temperature, is depicted in Figure S1 and detailed in the Experimental section. Initially, the thioether

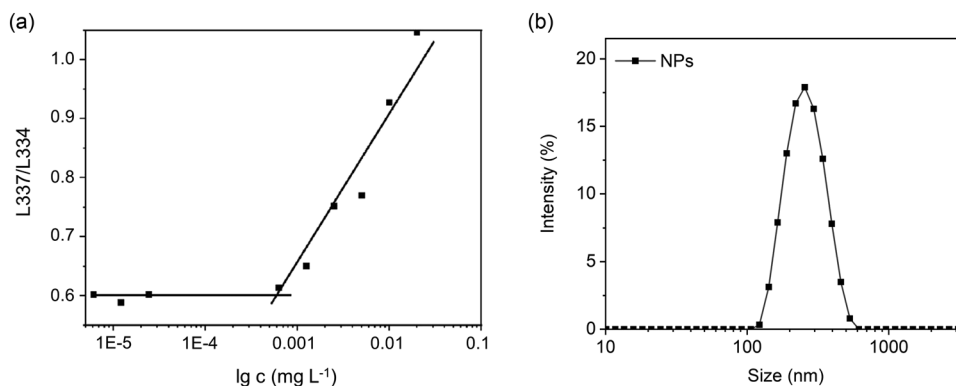
group is attached to glutamate through the esterification of Boc-MLG-OTBu with 2-(methylthio)ethanol. The MLG-NCA was then synthesized according to protocols in our previous report (24). The desired product, mPEG<sub>2k</sub>-*b*-PMLG, is ultimately obtained through the ring-opening polymerization of MLG-NCA utilizing mPEG<sub>2k</sub>-NH<sub>2</sub> as a macromolecular initiator in anhydrous DMF. By controlling the molar ratio of MLG-NCA and mPEG<sub>2k</sub>-NH<sub>2</sub> macromolecular initiator (17:1), the DP of PMLG is finally determined to be 15 by integrating the area of proton peaks f and i in Figure 1. GPC profiles showed that the average molecular weight of mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> is 6.1 kDa.

## 3.2 Self-assembly of mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub>

Some mPEG<sub>2k</sub>-based block copolymers exhibit an interesting behavior. As the temperature increases, the enhanced interaction between the hydrophobic fragments and the partial dehydration of the mPEG<sub>2k</sub> contributes to micelle accumulation. Eventually, this leads to a phase transition in the polymer solution, resulting in the formation of a gel (26,27). Our amphiphilic mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> could self-assemble into micelles or nanoparticles (NPs) in water. The critical micelle concentration (CMC) was determined to be 0.00083 mg·mL<sup>-1</sup> by using pyrene as fluorescence probe (Figure 2a). DLS tests have confirmed that these micelles maintain a stable 272 nm size at room temperature (Figure 2b).



**Figure 1:** <sup>1</sup>H NMR spectrum of mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> in mixture solvent of trifluoroacetic acid-*d* and CDCl<sub>3</sub>.



**Figure 2:** (a) CMC measurement determined by fluorescence assay of pyrene probe incubation with different concentrations of mPEG-*b*-PMLG<sub>15</sub> solution and (b) DLS profiles of the self-assembled NPs at 1 mg·mL<sup>-1</sup>.

### 3.3 Thermal-induced gelling and the mechanism

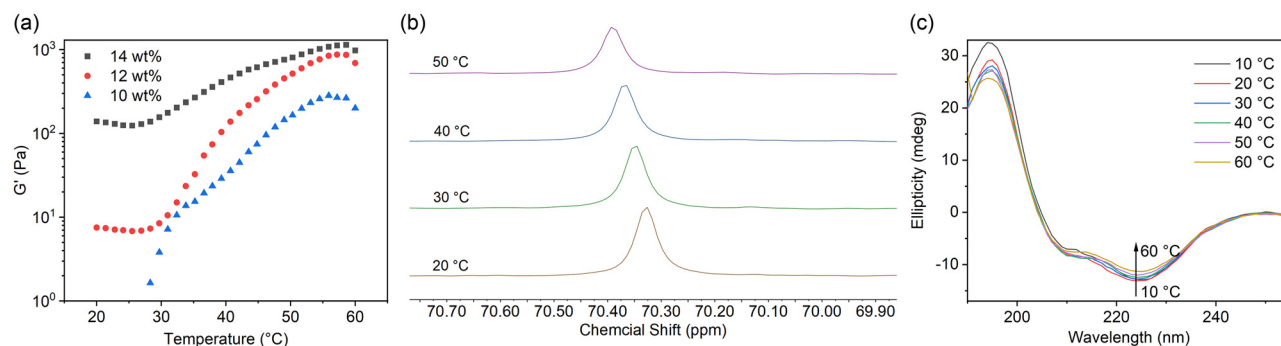
Dynamic rheological analysis of the aqueous solution of mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> was performed at the concentration ranging from 10 to 14 wt%. As shown in Figure 3a, when the temperature is lower than the phase transition temperature, the storage modulus  $G'$  of the polymer solution remains basically unchanged; At the vicinity of the phase transition temperature,  $G'$  suddenly increases, indicating that there is a solution transition to gel state, and with the increase of temperature,  $G'$  continues to increase. Specifically, the phase transition temperature was determined to be 33°C for 12 wt% polymer solution.

As depicted in Figure 3b, the resonance absorption peak of carbon in the mPEG methylene was observed at 70.3 ppm at 20°C. As the temperature gradually increased, this peak shifted toward the lower field and tends to broaden. This shift suggests that the mPEG segment experienced gradual dehydration as the temperature increased, accompanied by partial molecular immobilization. The

positive absorption peak at 195 nm and negative absorption peak at 225 nm in the CD spectra indicated a  $\beta$ -sheet structure of the PMLG<sub>15</sub> polypeptide (Figure 3c). The  $\beta$ -sheet structure became less at higher temperature as the intensity of peak at 225 nm gradually decreased when the temperature increased from 10°C to 60°C (Figure S2). Based on the aforementioned results, it can be inferred that the changes occurring in the mPEG segment and the secondary structure of the polyamino acid chain segments played a significant role in promoting the transition from a solution to a gel state as the temperature increased (25–28).

### 3.4 Oxidation responsiveness of mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub>

First, the invert test tube method was applied to confirm the phase transition temperature of mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub>, i.e., by observing whether the sample flows within 30 s through the inverted tube, if it does not flow, it is considered to



**Figure 3:** (a) The storage modulus ( $G'$ ) profiles of the mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> solution (10, 12, and 14 wt%) from 10°C to 60°C, (b) <sup>13</sup>C NMR spectra of polymer solution at temperature of 20°C–50°C, and (c) CD spectra of the mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> solution (0.05 mg·mL<sup>-1</sup>, 10°C–60°C).

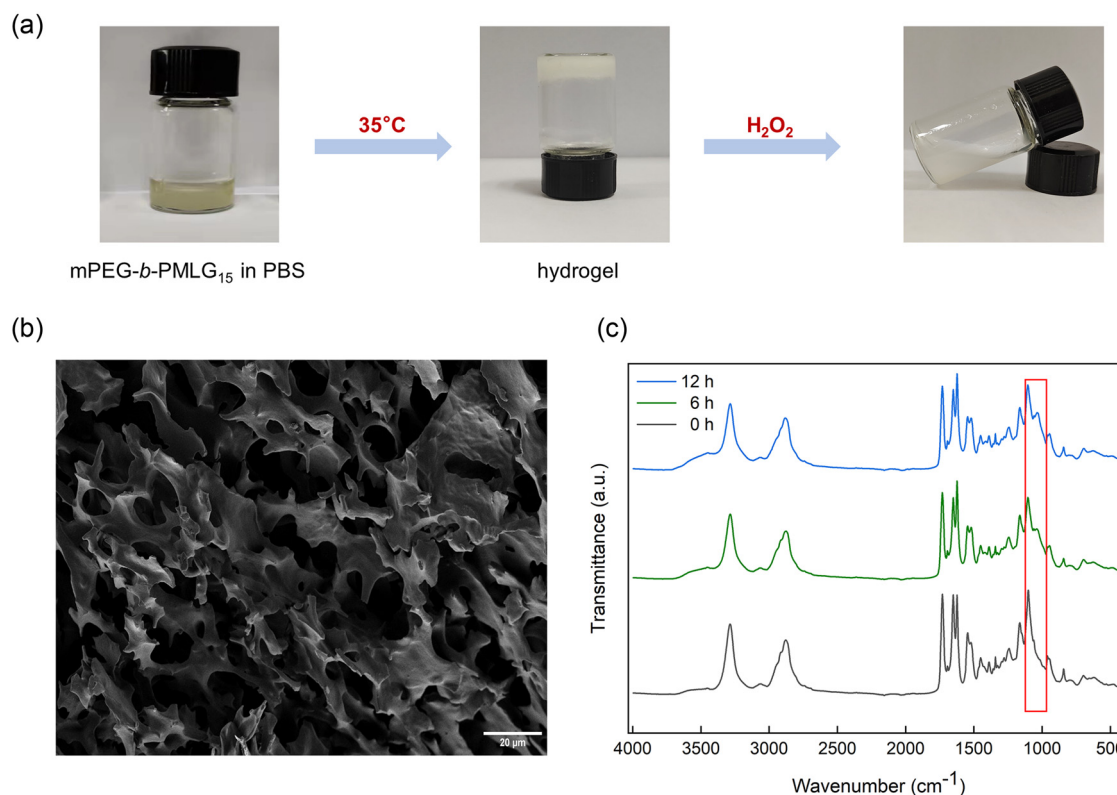
form a gel. As shown in Figure 4a, when the sample was inverted at 35°C, no flow occurred and hydrogel was formed. Scanning electron microscopy image exhibited the irregular pores of the hydrogel formed *in vitro* (Figure 4b).

H<sub>2</sub>O<sub>2</sub> is one of the key components of ROS generated during organism metabolism and exhibits relatively high stability compared to other ROS species. Therefore, we selected H<sub>2</sub>O<sub>2</sub> to create a high-concentration ROS environment to assess the gel's responsiveness to ROS. In the presence of H<sub>2</sub>O<sub>2</sub>, the hydrogel was destroyed and turned into liquid again (Figure 4a, right). As illustrated in the <sup>1</sup>H NMR spectra in Figure S3, the three peaks at 2.11, 2.70, and 4.21 ppm corresponding to the methyl and methylene near the thioether groups (labeled as a, b, and c) gradually disappeared after incubation with H<sub>2</sub>O<sub>2</sub> for 12 h. Concurrently, new peaks appeared at 2.68 (*a'*), 3.03 (*b'*), and 4.41 (*c'*) ppm, indicating the oxidation of the sulfur atom. Infrared spectra revealed a new absorption peak at 1,035 cm<sup>-1</sup> after oxidation, corresponding to the characteristic peak of the sulfoxide group, and the intensity of this characteristic peak significantly increased with prolonged oxidation time (Figure 4c). Therefore, the oxidative response mechanism is that the thioether group in the hydrophobic portion of mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> side chains is oxidized into hydrophilic

sulfoxide groups. As the hydrophilic properties of the polymer increase, its solubility also increases, which, in turn, accelerates the degradation of the hydrogel. This finding established a theoretical foundation for the elimination of ROS at inflammatory sites by mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> hydrogel and the protection of cells against oxidative damage.

### 3.5 Cell protection of gel in oxidized environment

The cytotoxicity of mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> and the hydrogel under oxidative conditions (10 mM H<sub>2</sub>O<sub>2</sub>) was evaluated using the MTT assay. As illustrated in Figure 5a, after a 24 h co-culture with the polymeric hydrogel and the oxidized products at concentrations ranging from 0.0156 to 1 mg·mL<sup>-1</sup>, the survival rate of L929 cells in the experimental group remained above 90%. This suggests that mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> block copolymers and their various oxidation products exhibit excellent cytocompatibility. In the presence of 0.25 and 0.125 mM H<sub>2</sub>O<sub>2</sub>, the cell survival rates of L929 were more than 80% with gel protection, significantly higher than that without gel protection (Figure 5b).



**Figure 4:** (a) Hydrogel formation at high temperature and disassociation in the presence of H<sub>2</sub>O<sub>2</sub>, (b) scanning electron microscope (SEM) image of the hydrogel formed by mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> solution at 35°C (scale bar: 20 μm), and (c) infrared spectra of hydrogel incubated with 100 mM H<sub>2</sub>O<sub>2</sub> for 0, 6, and 12 h, respectively.

ROS has high oxidation activity and can cause obvious damage and destruction to cells and tissues. Overexpression of ROS is the root cause of inflammation, including infectious inflammation (bacterial and viral infections) and non-infectious inflammation (such as rheumatoid arthritis), as well as a variety of senile degenerative diseases. Hydrogel formed by mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> solution could partly eliminate ROS and is potential for the treatment of oxidative stress-related diseases.

### 3.6 *In vivo* gelling of mPEG2k-*b*-PMLG<sub>15</sub>

A typical feature of temperature-sensitive hydrogels is that the aqueous solution of the polymer is rapidly converted into a hydrogel upon injection into the body. Therefore, SD rats were employed as an animal model to assess the subcutaneous gel process of the mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> hydrogel. The polymer solution was subcutaneously injected into the rats' backs, and after 15 min, the rats were euthanized. The back skin was then dissected, revealing the formation of the polymer gel within the subcutaneous tissue (Figure S4).

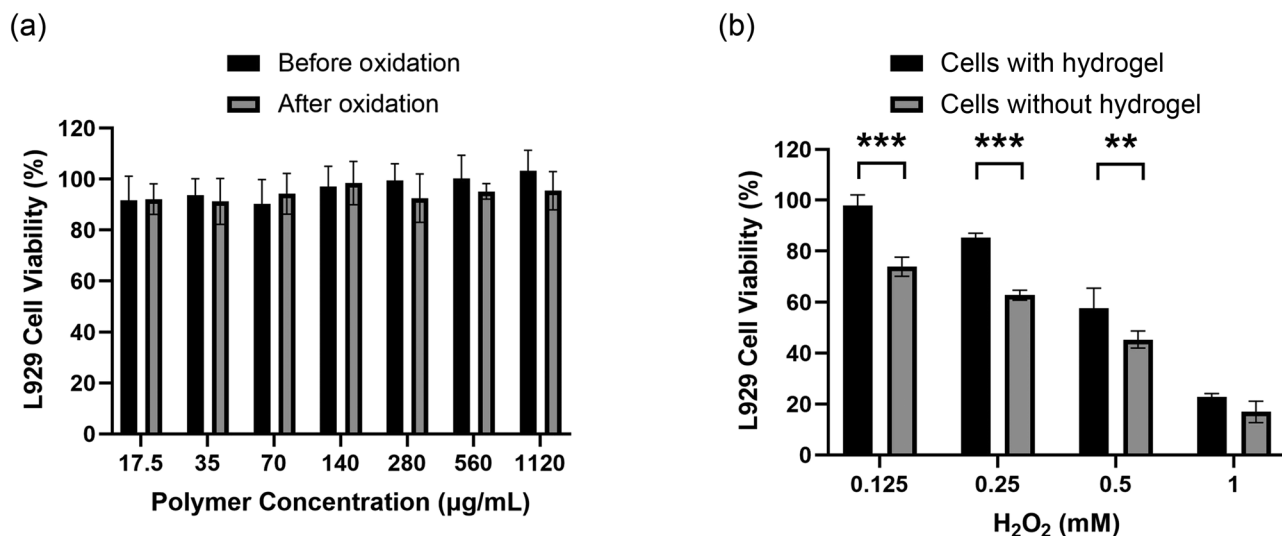
## 4 Discussion

The DP is one of the most important concepts in the field of polymer science. It has been reported that the self-assembled

nano-morphology of di-block amphiphilic copolymers might be spheres, cylinders, bicontinuous structures, lamellae, and enclosed membrane structures (vesicles or polymersomes), depending on the different length (or DP) ratio of the two blocks and solvent system (29–31). These different aggregates provide different potential applications in many fields.

Due to the different DPs of both PEG and polypeptides, our previously reported mPEG<sub>5k</sub>-*b*-PMLG<sub>40</sub> and the polymer in this study exhibited quite different properties (Table S1). The previous copolymers self-assembled into micelles of 68.3 nm, which were capable of encapsulating a high content of 9.4% DOX and achieving an enhanced anticancer efficacy in 4T1-tumor bearing mice (24). The long hydrophilic PEG<sub>5k</sub> segment contributed to the stability in blood circulation, while preventing the aggregation of micelles at elevated temperatures. Thus, mPEG<sub>5k</sub>-*b*-PMLG<sub>40</sub> solution could not be transformed to hydrogels. In contrast, lower-molecular mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> formed larger NPs with additional thermal sensitivity. With the increase of temperature, the PEG<sub>2k</sub> segment gradually dehydrated (Figure 3b), and the  $\beta$ -folding structure of PMLG<sub>15</sub> decreased (Figure 3c), which might promote the aggregation between these large micelles, limiting the mobility of aqueous solvent, and finally forming hydrogels (Figures 3a and 4a). Actually, curiosity caused us to synthesize shorter PEGylated polypeptides, mPEG<sub>2k</sub>-*b*-PMLG<sub>13</sub> and mPEG<sub>2k</sub>-*b*-PMLG<sub>11</sub>. The former can form hydrogels, whereas the latter cannot (data not shown).

By simply adjusting the DP, the mPEG-*b*-PMLG copolymers exhibited distinct properties, self-assembly behaviors,



**Figure 5:** (a) Cell survival rates of mouse fibroblasts cells (L929) after 24 h incubation with mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> solution or their oxidation products at different concentrations. It should be noted that the raw mPEG<sub>2k</sub>-*b*-PMLG solution formed hydrogel at 37°C, and (b) cell viability of L929 cells cocultured with 12 wt% hydrogel for 24 h in the presence of 0.125, 0.25, 0.5, and 1 mM H<sub>2</sub>O<sub>2</sub> in DMEM. Data are shown as mean  $\pm$  SD ( $n = 3$ ), \*\*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

and thus had different potential biomedical applications. This is the magic and charm of polymer science. Our bi-stimulus-responsive PEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> hydrogels have potential applications in hydrophilic drug delivery and in wound care dressings in the future.

## 5 Conclusion

In summary, we successfully synthesized mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> block copolymers. These polymers can self-assemble into nanosized micelles in an aqueous solution, subsequently forming a hydrogel when the temperature raised. The gelation mechanism was attributed to the partial dehydration of PEG segment and the secondary structure change of polypeptide block as temperature increasing. *In vitro* experiments demonstrated that both of the polymeric hydrogel and the post-oxidized product exhibited negligible cytotoxicity. The survival rate of L929 cells incubated with the polymeric hydrogel exceeded 90% at H<sub>2</sub>O<sub>2</sub> concentrations of 0.125 mM, effectively mitigating cellular damage in the presence of elevated H<sub>2</sub>O<sub>2</sub> levels. In addition, a regular-shaped and solid gel was formed within 15 min injection of mPEG<sub>2k</sub>-*b*-PMLG<sub>15</sub> solution in rat. This injectable hydrogel, based on essential human amino acids, holds promise for applications in the localized, on-demand release of drugs, and cellular protection in areas enriched with ROS, such as tumor site and inflammatory region.

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**Author contributions:** Yingying Ji: writing – original draft, experimenting, data curation; Yitao Sun: polymer synthesis and cellular experiment; Yu Zhang: funding acquisition, project administration; Hongyu Zhang: methodology; Pan He: writing – review and editing, supervision, funding acquisition.

**Conflict of interest:** The authors declare that they have no conflict of interest.

**Ethical approval:** Female SD rats were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. The animal experimental protocols were approved by Ethics Committee of Changchun Institute of Applied Chemistry (No. 2022-0082).

**Data availability statement:** Original data can be requested from the corresponding authors.

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