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Synthesis and Characterisation of Core Cross-linked Micelles for Drug Delivery

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Abstract— **A novel redox-sensitvie cross-linked micelles based on disulfide-linked polymer were prepared and characterized as potential carrier for drug delivery. In this experiment N,N-bis(acrylate) cystamine (BAC) served as cross-linker, poly(γ-benzyl-L-glutamate) (PBLG) and polyethylene glycol (PEG) methyl ether methacrylate acted as comonomers. The molecular structure and characteristics of the cross-linked micelle was confirmed by ^{1}H NMR and FT-IR. The copolymer was able to self-assembled into micelles, and the micellar nanostructure was investigated by DLS and TEM. The cross-linked micelles was designed to degraded into individual linear short chains in the presence of glutathione (GSH) by the cleavage of the disulfide linkages from the cross-linker BAC. All the results showed that the designed cross-linked polymeric micelles may be a promising carrier for drug delivery in intracellular reducing environment.**

Index Terms—**Cross-linked Micelles, Redox-sensitive, controlled release, anticancer drugs**

INTRODUCTION

Polymeric micelles prepared from self-assembling amphiphilic block copolymers in aqueous solution have been represented a very promising drug delivery system in recent years[1-4]. This kind of micelle consisted of hydrophobic core as a container for hydrophobic drugs, and the hydrophilic outer shell as a hydration barrier with good biocompatibility, improved colloidal stability, and less side effects of drugs on healthy cells[5-7].

However, the unexpected drug release caused by micellar instability hinders the effective delivery of their payloads at aimed sites of action, and may cause serious toxicity problems, which extremely limit their in vivo application. To stabilize polymeric micelles for in vivo applications block copolymers can be chemically cross-linked in the micellar core. Therefore, degradable cross-linked polymeric drug carriers are introduced to improve the drug delivery and ensure elimination of drug side effects[8-11].

Degradable cross-linked micelles with redox stimulus sensitivity demonstrated effective drug release behavior by the large difference in reducing potential between the tumor tissues and normal tissues, with at least 4-fold higher concentrations of GSH in the tumor tissues over normal tissues[12-14]. Therefore, with the aim to achieve targeted and efficient drug delivery, redox-sensitive cross-linked polymeric micelles are of particular interest for targeted intracellular drug delivery[15-18].

In this experiment, our object is to design and prepared a novel redox-sensitive core cross-linked micelle, to obtain a biocompatible, degradable, robust, and smart drug carrier. In our architecture, the redox-sensitive micelles comprised an interior polymer network formed by PBLG and N,N-bis(acrylate) cystamine (BAC) as cross-linker, and a polyethylene glycol (PEG) corona to provided colloidal stability. The structural characterization data were confirmed by analyses using ^{1}H NMR and FTIR spectra. The micelles formed from the core cross-linked polymer were stable in physiological condition by the investigation of TEM and DLS.

EXPERIMENTAL SECTION

Materials

PEG methyl ether methacrylate was obtained from Sigma-Aldrich. 2-Hydroxyethyl disulfide and acryloyl chloride were purchased from Alfa and used as received. Glutathione (GSH) was purchased from BIOSHARP (Japan). 2,2-azobisisobutyronitrile (AIBN) was obtained from Kemio Chemical Reagent Company (Tianjing, China). Triphosgene (99%, Aladdin) was recrystallized before use.

Characterization

^{1}H NMR spectra of cross-linked micelles and related copolymers were obtained using a Bruker 400-MHz spectrometer with deuterated chloroform ($CDCl_3$-d) as solvent, and tetramethylsilane (TMS) as an internal standard. FTIR data were gathered in solid state on a PE Spectrum One FTIR spectrophotometer under ambient from 400 to 4000 cm^{-1}, utilizing a resolution at 4 cm^{-1} resolution. The micelle size (D_h) and distribution (PDI) were determined by DLS in aqueous solution using a Malvern Zetasizer Nano-ZS90 apparatus Morphologies of micelles were investigated by transmission electron microscopy (Hitachi H-600, Japan).

Synthesis of N,N-bis(acrylate) cystamine (BAC)

2-Hydroxyethyl disulfide and triethylamine were dissolved in methylene chloride (60 mL), and cooled in ice-water bath for 30 min. Acryloyl chloride was added dropwise slowly, and the reaction mixture was cooled in ice-water bath and stirred at 0 ^oC for 1 h and then at room temperature for 12 h. The filtered solution was washed with $NaHCO_3$ and water, and distilled under vacuum. A purified product was easily obtained by passing through a silica gel column.

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Synthesis of allyl-terminated PBLG

In a typical experiment, Bz-L-GluNCA (0.78 g), allylamine (6.0 μL), and 2.7 mL of anhydrous DMF were introduced into a dry Schlenk flask. After degassed by three freeze-thaw cycles, the solution was stirred for 24 h at room temperature under N_2 atmosphere. The polymer was precipitated in diethyl ether and dried under vacuum.

Preparation and characterization of cross-linked polymeric micelles

A typical procedure for preparing the cross-linked micelles is as following: allyl-terminated PBLG (0.12 g), PEGMA (1.20 g), BAC (0.06 g) and AIBN (0.05 g) were dissolved in 150 mL of toluene in a dried single-necked flask. The reaction mixture was heated from ambient temperature to 80 ^{o}C with vigorous stirring for 12 h. The obtained cross-linked copolymer was precipitated in a large diethyl ether, and dried in a vacuum overnight.

The polymeric micelles were prepared by a dialysis method. In a typical experiment, 10 mg cross-linked copolymer was dissolved in 2 mL of DMF. Under gentle stirring, the solution was dropwisely added to 5 mL of double-distilled water in 1 h. The solution was transferred to a dialysis bag and dialyzed against deionized water at 25 °C for 24 h to remove the organic solvents with frequent replacement of deionized water. To obtain micelle powder, the micellar solution was frozen and lyophilized. The size and morphology of the micelles were investigated by DLS and TEM.

RESULTS AND DISCUSSION

Synthesis and characterization of cross-linked copolymer.

In this work, a new type of disulfide-bonded cross-linked copolymers was prepared with PBLG, PEGMA, and the disulfide-bonded cross-linker BAC in free radical copolymerization. AIBN was used as initiator, and toluene was acted as solvent. By a dialysis method, the prepared cross-linked copolymer formed micelles at room temperature and could remain stable due to the formation of hydrogen bonds between hydrophilic PEG outer shell and water molecules as a hydration barrier. The hydrophobic PBLG enhanced the biocompatibility and loading capacity of drug, and the incorporation of hydrophilic PEGMA improved the colloidal stability of micelles. In the synthesis of cross-linked PEG-polypeptide hybrid micelles, the sum PEGMA + PBLG, 1 g, and mass ratios of PEGMA to PBLG (98/2) were used. The feeding concentrations of the initiator (AIBN) and cross-linker (BAC) were set to a weight ratio of 4 wt %. The typical recipes and tested results were shown in Table 1. **Table 1. The feed composition and colloidal data of cross-linked micelles**

Sample	PEGMA (mg)	PBLG (mg)	D_{TEM} (nm)	D_h[a] (nm)	PDI[b]
Cross-linked Micelle	980	20	103	122	0.151

a The hydrodynamic diameter (D_h) was determined in phosphate buffer of 7.4 at 25 °C by DLS.
b PDI, polydispersity index of the particle size.

The chemical structure of the synthesized cross-linked copolymer was confirmed by ^{1}H NMR. The ^{1}H NMR spectrum of copolymer micelle (in CDCl3 with 15% TFA) was depicted in Figure 1. The resonance signals at 4.09-4.30 (c), 3.6-3.9 (d), 3.5-3.6 (a', e), 1.8-2.1 (a, b), and 0.9 ppm (b') were belong to the glycol unit (c, d), methyl end group (e) in PEG block, –CH_2– (a, a', b), and –CH_3– (b') in polymer backbone. As seen in Figure 1, the disappearance of vinyl group in PLBG indicated the successfully synthesis of the cross-linked copolymer.

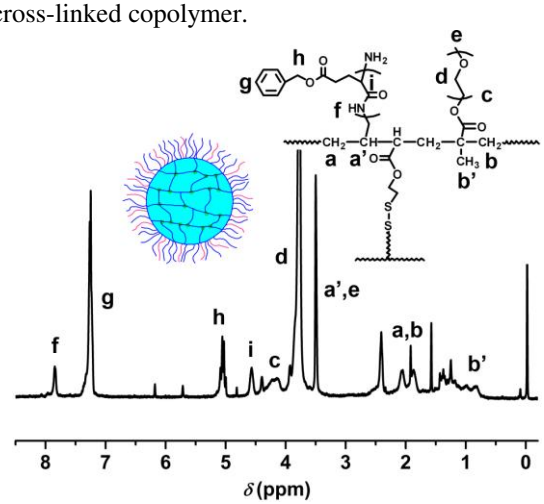
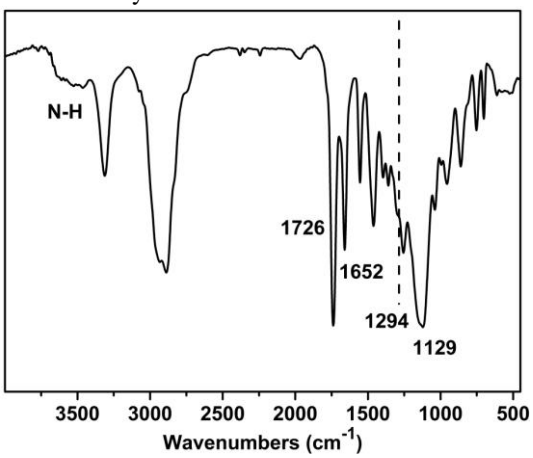


Figure 1. ^{1}H NMR spectrum of cross-linked copolymer in a $CDCl_3$/TFA mixture with 15%TFA.

The cross-linked copolymer was also characterized by FTIR as shown in Figure 2. In the spectrum of cross-linked polymer, the typical vibrational band of C=O and C−S appeared at 1726 cm^{-1} and 1294 cm^{-1} for cross-linker (BAC). Amide I bands around 1652 cm^{-1} and the absorbance of the N−H around 3400 cm^{-1} were distinctively observed for PBLG blocks. For the cross-linked copolymer, a strong absorption band 1129 cm^{-1} assigned to the C-O group in glycol unit of PEG, indicating the completion of copolymerization by crosslinker.



Figuer 2. The FTIR spectrum of cross-linked copolymer.

Micelle formation

Micecles of the synthesized cross-linked copolymer were prepared by dialysis method. Dynamic light scattering (DLS) measurements showed that cross-linked copolymers formed micelles with sizes of 85 nm (Figure 3). The micelles both had a narrow unimodal distribution with a PDI of 0.12 indicating the assembled micelles a good physical performance in aqueous solution. TEM micrograph revealed

that these micelles had a spherical morphology with an average size of about 70 nm. The smaller size observed by TEM as compared to that determined by DLS is most likely due to shrinkage of the PEG shell.

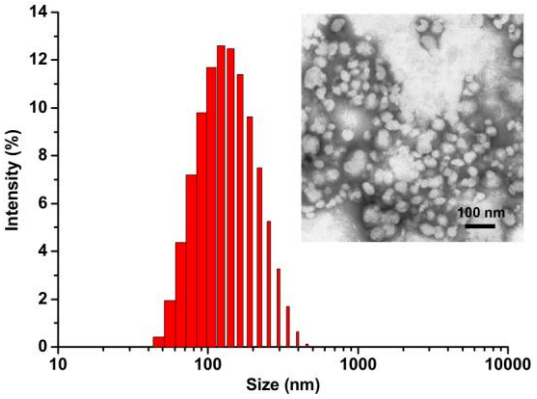


Figure 3. Size distribution and TEM photo of copolymer micelle.

CONCLUSION

In summury, a novel cross-linked micelles based on disulfide-linked polymer were prepared and demonstrated for drug delivery. The molecular structures and characteristics of the cross-linked micelles were confirmed by ^{1}H NMR and FT-IR. The obtained micelles possessed uniform, spherical shape with the average hydrodynamic diameter smaller than 100 nm and narrow size distribution. In general, all the results showed that the designed cross-linked micelles with good biocompatibility and enhanced stability are promising drug delivery systems for targeting therapy.

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