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Biocompatible Polymeric Analogues of DMSO Prepared by Atom Transfer Radical Polymerization

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Abstract

The synthesis of a sulfoxide-based water-soluble polymer, poly(2-(methylsulfinyl)ethyl acrylate) (polyMSEA), a polymeric analogue of DMSO, by atom transfer radical polymerization (ATRP) is reported. Well-defined linear polymers were synthesized using relatively low amounts of copper catalyst (1000 or 100 ppm). Two types of star polymers were synthesized by either an “arm-first” approach or a “core-first” approach using a biodegradable β -cyclodextrin core. The glass transition temperatures of both the linear polymer (16 °C) and star polymer (32 °C) were determined by differential scanning calorimetry (DSC). The lower critical solution temperature (LCST) of poly(MSEA) was estimated to be ca. 140 °C by extrapolating the LCST of a series of copolymers with NIPAM. Cytotoxicity tests revealed that both the linear and star polymers have low toxicity, even at concentrations up to 3 mg/mL.

Graphical abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bio-mac.6b01553. Details of star polymer synthesis, DSC traces, determination of reactivity ratios and DLS traces of polyMSEA-co-polyNIPAM copolymers are reported (PDF)

INTRODUCTION

Biocompatible polymers are used in a wide range of therapeutic applications, including drug delivery,¹ siRNA delivery,² gene delivery,³ vaccination,⁴ and tissue engineering.⁵

Poly(ethylene glycol) (PEG) is among the most widely used polymers in biomedical applications due to its hydrophilicity, water-solubility, and chemical stability of the ether groups. Its neutral charge prevents interactions with negatively charged cell membranes, or opsonins that enhance phagocytosis and polymer clearance.⁶ PEGylated species can circulate longer in the bloodstream.⁷ However, the conjugation of monofunctional PEG to biorelevant molecules may result in the formation of heterogeneous products due to the presence of bifunctional PEG impurities.⁷ Low molecular weight PEG may aggregate at elevated temperatures due to its lower critical solution temperature (LCST). In addition, it has been recently reported that the immunogenicity of PEG may result in inefficient drug delivery and severe immune reactions.⁷ These limitations have motivated studies on the preparation and evaluation of other types of water-soluble, biocompatible polymers, with diverse chemistry to replace PEG. Poly(2-oxazoline)s, also synthesized by ring-opening polymerization, are potential alternatives to PEG. However, depending on substituents, they may exhibit limited solubility in water.⁸

It is interesting to explore synthesis of biocompatible polymers by reversible deactivation radical polymerizations (RDRP) procedures, such as atom transfer radical polymerization (ATRP),^{9,10} reversible addition–fragmentation chain transfer (RAFT) polymerization,¹¹ and nitroxide-mediated polymerization (NMP).¹² RDRP was already employed to prepare some PEG-alternatives, such as poly(oligo(ethylene oxide) methyl ether methacrylate),^{13,14} polycarboxybetaines,¹⁵ polyacrylamides,¹⁶ and polyvinylpyrrolidone.¹⁷ Recently, several procedures were developed to reduce concentration of ATRP catalyst, including activator regeneration by electron transfer (ARGET) ATRP,¹⁸ supplemental activator and reducing agent (SARA) ATRP,^{19,20} initiators for continuous activator regeneration (ICAR) ATRP,²¹ photoATRP,^{22,23} and *e*-ATRP.²⁴ These systems have also been successfully applied to aqueous media.²² Polymers with various architectures, such as hyperbranched polymers,²⁵ star polymers,^{26,27} nanogels,²⁸ and other topologies,^{29,30} have been prepared under such conditions.

Previously, the synthesis of polymers based on sulfur-containing monomers, disulfide dimethacrylate,^{13,14,29} 2-(methylthio)ethyl acrylate (MTEA), and 2-(methylthio)ethyl methacrylate (MTEMA),³¹ was reported. MTE(M)A was also converted to tertiary sulfonium species stable in an aqueous environment. The diblock copolymers of PEG with sulfonium segments were biocompatible up to 50 $\mu\text{g/mL}$. They also efficiently formed polyplexes with siRNA for gene knockdown.³¹

Dimethyl sulfoxide (DMSO) is a neutral uncharged molecule used as a transdermal delivery enhancer,³² polymerase chain reaction inhibitor,³³ cryoprotectant,³⁴ and in veterinary medicine.³⁵ The biocompatibility of DMSO is largely due to the presence of the polar aprotic methyl sulfoxide group.³² Inspired by these unique properties of DMSO, we aimed to synthesize a polymeric analogue of DMSO by ATRP with high water-solubility and low cytotoxicity.^{36–38} In this paper, the polymeric analogue of DMSO was prepared by oxidation

of well-defined poly(2-(methylthio)ethyl acrylate) (polyMTEA) and also directly from the sulfoxide containing monomer, 2-(methylsulfinyl)ethyl acrylate (MSEA), formed via oxidation of MTEA. Well-defined linear polyMSEA with low dispersity (<1.3) was synthesized by ARGET ATRP in the presence of 100 ppm copper catalyst. In addition, two well-defined star polymers^{39,40} were prepared, one using a “core-first” approach with a biodegradable β -cyclodextrin core and the other one by an “arm-first” approach with divinylbenzene as cross-linker. The LCST of pure polyMSEA (ca. 140 °C) was estimated from the solution aggregation points of copolymers of MSEA and NIPAM with different compositions. The cytotoxicity of neutral water-soluble linear and star-shaped polyMSEAs was evaluated using human embryonic kidney cells (HEK 293) at concentrations up to 3 mg/mL. These new polymers could potentially be considered as an alternative to PEGylation approach in some biomedical applications.

EXPERIMENTAL SECTION

Materials

2-(Methylthio)ethanol (99%) was purchased from Alfa Aesar. Hydrogen peroxide solution (30%) was purchased from Fisher Scientific. Acrylic acid (99%), tin(II) 2-ethylhexanoate ($\text{Sn}^{\text{II}}(\text{EH})_2$, 95%), methyl acrylate (99%), *n*-butyl acrylate (99%), poly(ethylene glycol) methyl ether acrylate (OEGA, average $M_n = 480$), *N*-(3-(dimethylamino)propyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 99%), *N,N*-dimethylamino pyridine (DMAP, 99%), copper bromide (99.99%), ascorbic acid, (AA, 99%), divinylbenzene (DVB, technical grade, 80%), and ethyl α -bromoisobutyrate (EBiB, 98%) were purchased from Sigma-Aldrich. Acrylic acid was distilled under vacuum before use. All acrylate monomers were passed through basic alumina columns before use. Tris(2-pyridylmethyl)amine (TPMA) was synthesized according to previous procedures.⁴¹ All solvents and other chemicals are of reagent quality and were used as received unless special treatments discussed below were applied.

Instrumentation

^1H nuclear magnetic resonance (^1H NMR) measurements were performed on a Bruker Avance 300 MHz spectrometer. Molecular weight and molecular weight distribution (M_w/M_n) were determined by a combination of gel permeation chromatography and multiangle light scattering (GPC-MALS). The GPC-MALS used a Waters 515 HPLC pump, Wyatt Optilab refractive index detector, Wyatt DAWN HELEOS-II multiangle light scattering detector and PSS columns (Styrogel 10², 10³, and 10⁵ Å) with either 40 °C pure THF or 50 °C 50 mM LiBr DMF solution as eluent phase at flow rate of 1 mL/min. Differential scanning calorimetry (DSC) was performed on a TA Instrument QA-2000 three times for each sample, using the following procedures: (1) hold at –80 °C for 2 min; (2) heat to 130 °C at rate of 10 °C/min; (3) hold for 2 min; (4) cool down to –80 °C. Temperature-dependent dynamic light scattering (DLS) was performed on a Malvern Zetasizer Nano ZS.

Synthesis of 2-(Methylsulfinyl)ethyl acrylate (MSEA)

2-(Methylthio)ethyl acrylate (MTEA) was synthesized according to the previously reported procedure.³¹ In a typical oxidation procedure, 5 g MTEA (1 equiv) was added to a 25 mL

round-bottom flask sealed with rubber stopper. The flask was kept in an ice bath and purged with N₂ then 3.77 g hydrogen peroxide solution (1.1 equiv) was slowly injected into the flask at rate of 50 μ L/min. The reaction was allowed to stir for 24 h and was then stopped by adding 50 mL deionized water. The aqueous solution was washed 3 times with 100 mL dichloromethane. The organic phase was collected and dried over magnesium sulfate. Excess solvent was removed under vacuum to give 4.5 g pure product of MSEA (yield 81.2%). ¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) = 6.38 (1H, dd, CHH=CH), 6.19 (1H, q, CHH=CH), 5.98 (1H, dd, CHH=CH), 4.49 (2H, m, C=OOCH₂CH₂), 3.15 (1H, m, CH₂CHHS=O), 2.99 (1H, m, CH₂CHHS=O), 2.60 (3H, s, S=OCH₃).

Synthesis of Linear polyMSEA

The polymerization of MSEA was carried out using A(R)GET ATRP. In a typical AGET procedure, 8.6 mg EBiB (1 equiv), 0.5 g MTEA (70 equiv) 0.69 mg copper(II) bromide (0.07 equiv), 1.9 mg TPMA (0.15 equiv), 0.1 mL DMF, and 2.9 mL DMSO were mixed in a sealed 10 mL Schlenk flask equipped with a stirring bar. The Schlenk flask was degassed by three freeze–pump–thaw cycles. The flask was allowed to warm up to room temperature and placed in an oil bath thermostated at 35 °C. Deoxygenated ascorbic acid (AA) solution (5.4 mg in 0.1 mL DMSO) was injected into the flask under N₂ purge to trigger the polymerization, and an initial sample ($t = 0$) was collected by syringe. Samples were taken periodically to measure conversion via ¹H NMR and molecular weight via GPC. The polymer was precipitated in ether, purified by dialysis (MWCO = 100–500 Da) against water, and dried by lyophilization. In a typical ARGET procedure, 8.6 mg EBiB (1 equiv), 0.5 g MTEA (70 equiv) 0.04 mg copper(II) chloride (0.007 equiv), 0.2 mg TPMA (0.015 equiv), 0.1 mL DMF, and 2.9 mL DMSO were mixed in a sealed 10 mL Schlenk flask equipped with a stirring bar. The Schlenk flask was degassed by three freeze–pump–thaw cycles. The flask was allowed to warm up to room temperature and placed in an oil bath thermostated at 35 °C. Deoxygenated ascorbic acid (AA) solution (1.1 mg in 0.1 mL DMSO) was injected into the flask under N₂ purge intermittently over 5 h, and an initial sample ($t = 0$) was collected by syringe. Samples were taken periodically to measure conversion via ¹H NMR and molecular weight via GPC.

Measurement of Reactivity Ratios

The reactivity ratio were determined using the Kelen-Tüdös approach.^{42,43} For the MSEA/MA pair (monomer 1: MSEA, monomer 2: MA), four ATRP reactions were conducted with the ratio of reagents [MSEA]₀/[MA]₀/[EBiB]₀/[AA]₀/[CuBr₂]₀/[TPMA]₀ = $x/(70 - x)/1/0.7/0.07/0.15$ in DMSO at the same concentration (1 M of all monomers), $x = 60, 45, 25, 10$. For the MSEA/*n*BA pair (monomer 1: MSEA, monomer 2: *n*BA), four ATRP reactions were conducted with [MSEA]₀/[*n*BA]₀/[EBiB]₀/[AA]₀/[CuBr₂]₀/[TPMA]₀ = $x/(70 - x)/1/0.7/0.07/0.15$ in DMSO at the same concentrations as the above example, $x = 60, 45, 35, 10$. For the MSEA/OEGA pair (monomer 1: MSEA, monomer 2: OEGA), four different ratios of [MSEA]₀/[OEGA]₀/[EBiB]₀/[AA]₀/[CuBr₂]₀/[TPMA]₀ = $x/(70 - x)/1/0.7/0.07/0.15$ in DMSO were carried out under the same concentrations as the above example, $x = 60, 45, 35, 10$. For the MSEA/NIPAM pair (monomer 1: MSEA, monomer 2: NIPAM), four different ratios of [MSEA]₀/[NIPAM]₀/[EBiB]₀/[AA]₀/[CuBr₂]₀/[TPMA]₀ = $x/(70 - x)/1/0.7/0.07/0.15$ in DMSO were carried out under the same concentrations as the

above example, $x = 60, 45, 35, 25$). The conversion of monomers was measured by ^1H NMR. All reactions were stopped within 15 min to keep conversion low.

Synthesis of Star Polymer (polyMSEA) $_n$ -polyDVB by “Arm-First” Approach

The polyMTEA macroinitiators were synthesized by ARGET ATRP. In a typical procedure, 0.222 g EBiB (1 equiv), 10 g MTEA (60 equiv), 17.8 mg CuBr_2 (0.07 equiv), and 49.7 mg TPMA (0.15 equiv) were mixed in a solution of 11 mL acetone and 0.5 mL DMF in a sealed 50 mL Schlenk flask equipped with a stirring bar. The Schlenk flask was degassed by three freeze–pump–thaw cycles. The flask was allowed to warm up to room temperature and placed in an oil bath thermostated at 35 °C. Deoxygenated ascorbic acid (AA) solution (140 mg in 0.5 mL DMF) was injected into the flask under a N_2 purge to activate the polymerization, and an initial sample ($t = 0$) was collected by syringe. Samples were taken periodically to measure conversion via ^1H NMR and molecular weight via GPC. The polymer with $M_n = 3000$ was isolated by precipitation in methanol and dried under vacuum.

The polysulfide star (polyMTEA) $_n$ -polyDVB was synthesized by ARGET ATRP using the aforementioned polyMTEA-Br as macro-initiator. In a typical procedure, 470 mg polyMTEA-Br (1 equiv), 222 mg DVB (14 equiv), 0.16 mg CuBr_2 (0.01 equiv), and 3.5 mg TPMA (0.1 equiv) were mixed in a solution of 4.5 mL anisole and 0.1 mL DMF in a sealed 10 mL Schlenk flask equipped with a stirring bar. The Schlenk flask was degassed by three freeze–pump–thaw cycles. The flask was allowed to warm up to room temperature and then placed in an oil bath thermostated at 90 °C. A deoxygenated solution of 9.9 mg $\text{Sn}(\text{EH})_2$ in 0.5 mL anisole was injected into the flask under N_2 purge to reduce the cupric complex and trigger the polymerization, and an initial sample ($t = 0$) was collected by syringe. Samples were taken periodically to measure conversion via ^1H NMR and molecular weight via GPC-MALS. The final polymer was isolated by precipitation in ether to remove nonpolymerized DVB and purified by dialysis (MWCO = 10 000 Da) against THF and dried under vacuum.

The polysulfoxide star, (polyMSEA) $_n$ -polyDVB, was synthesized by postoxidation. In a typical procedure, 0.1514 g (polyMTEA) $_n$ -polyDVB (1 equiv per MSEA repeating unit) was dissolved in 1 mL deoxygenated THF in a sealed 10 mL round-bottom flask equipped with a stirring bar. 121.3 mg hydrogen peroxide solution (1.3 equiv) was slowly injected at rate of 50 $\mu\text{L}/\text{min}$ and allowed to react for 72 h, and then the product was precipitated by addition of the solution to 10 mL THF and purified by dialysis (MWCO = 10 000 Da) against deionized water and dried under vacuum to give pure (polyMSEA) $_n$ -polyDVB. The molecular weight of star polymers was analyzed by GPC-MALS.

Synthesis of β -CD-14Br ATRP Initiator

The synthesis of macroinitiator was done according to previous report.⁴⁴ In a typical procedure, 10 g of β -cyclodextrin (β -CD, 8.81 mmol) was dried under vacuum overnight and placed in 250 mL round-bottom flask. The β -CD was dissolved in 100 mL of anhydrous 1-methyl-2-pyrrolidone (NMP) and the flask was placed in an ice bath. 2-Bromoisobutryl bromide (BriBB) (27 mL, 1.2 equiv to $-\text{OH}$) was dissolved in anhydrous NMP (50 mL) and slowly added to the β -CD solution. The solution temperature was allowed to warm up to room temperature and the reaction was stirred for 1 day. The dark brown solution was

dialyzed against distilled water (MWCO = 500) for 1 week. The remaining product was concentrated at reduced pressure and crystallized in cold hexane to obtain a pale yellow solid product. The ^1H NMR spectrum of the macroinitiator in CDCl_3 is shown in Figure S5. The peak at ~ 2.0 ppm was from the solvent of NMP and the methyl groups on the bromo initiator sites. The functionality was calculated by equation: $\delta_{\text{ini}}/(\delta_{\text{H1}} \times 3 \times 7)$. The functionality of the macroinitiator corresponded to 14 initiator sites per β -CD molecule with a purity of 95%.

Synthesis of Star Polymer (polyMSEA)_n-(β -CD) by “Core-First” Approach

The “grafting from” polymerization of MSEA was carried out using ARGET ATRP. In a typical procedure, 10 mg β -CD macroinitiator (1 equiv), 0.5 g MSEA (70 equiv), 1.38 mg copper(II) bromide (0.14 equiv), 6.4 mg TPMA (0.5 equiv), and 3 mL DMF were mixed in a sealed 10 mL Schlenk flask equipped with a stirring bar. The Schlenk flask was degassed by three freeze–pump–thaw cycles. The flask was allowed to warm up to room temperature. Deoxygenated ascorbic acid (AA) solution (5.4 mg in 0.1 mL DMF) was injected into the flask under N_2 purge to trigger the polymerization, and an initial sample ($t = 0$) was collected by syringe. Samples were taken periodically to measure conversion via ^1H NMR and molecular weight via GPC MALS.

Determination of the LCST of polyMSEA

The polymers for LCST determination were prepared by copolymerizing different ratios of MSEA with *N*-isopropylacrylamide (NIPAM) by free radical polymerization (FRP). In a typical procedure, 100 mg MSEA (12 equiv), 104.7 mg NIPAM (18 equiv), and 8.53 mg AIBN (1 equiv) were dissolved in 2 mL DMF in a 10 mL Schlenk flask equipped with a stirring bar. The flask was sealed and degassed by three freeze–pump–thaw cycles. The flask was allowed to warm up to room temperature and placed in an oil bath thermostated at 70 °C. All reactions were allowed to reach ca. 99% conversion and the copolymers were precipitated by addition of the reaction mixture to ether. The final product was collected and dried in vacuum oven overnight. For DLS analysis, 30 mg of the as-dried copolymer was dissolved in 3 mL deionized water and passed through a 0.2 μm PTFE filter before tests.

Cytotoxicity Test

Human Embryonic Kidney Cells (HEK 293) were grown in a 96 well tissue culture plate at a density of 10 000 cells/well for 24 h. 100 mg/mL solutions of the linear polyMSEA and (polyMSEA)_n-polyDVB were prepared by mixing with 1× PBS which was then added to the tissue culture plate at concentrations of 10, 30, 100, and 300 μg . All concentrations were tested in triplicate. The polymer was then incubated with the HEK 293 cells for 48 h at 37 °C in the presence of 5% CO_2 . After 48 h, ATP Cell Titer Glo Assay was used and luminescence was measured and quantitated as a percentage of the control wells.

RESULTS AND DISCUSSION

Synthesis of MSEA Monomer and polyMSEA by ATRP

The synthetic route for MSEA is shown in Scheme 1. The monomer 2-(methylthio)ethyl acrylate (MTEA) was synthesized by an EDC catalyzed esterification procedure detailed in

the previous work.³¹ The sulfoxide containing monomer 2-(methylsulfinyl)ethyl acrylate (MSEA) was then prepared by oxidation with 1.1 equiv of hydrogen peroxide in an aqueous solution. The hydrogen peroxide solution was injected into MTEA (liquid state) at feed rate of 50 $\mu\text{L}/\text{min}$ in order to avoid potential side reactions caused by heat release. The oxidation was complete and no further oxidation to sulfone occurred, as confirmed by the absence of the NMR peaks of methine protons next to a sulfone group at ~ 3.6 ppm.⁴⁵

The ^1H NMR spectrum of MSEA in $\text{DMSO}-d_6$ is shown in Figure 1. The peak integration ratio of **a/b/a'/c/d/e** equals 1.00/0.98/1.01/2.04/2.06/3.02 confirming the high purity of the synthesized monomer. The **c** protons and **d** protons split into two peaks due to two prochiral carbons next to a chiral sulfur atom. The peak splitting of **c** protons is less pronounced as they are farther from the chiral sulfur than **d** protons. The $\text{DMSO}-d_6$ peak is located at 2.5 ppm and is negligible due to high concentration of the monomer.

Free radical polymerization (FRP) of MSEA was previously reported.^{46,47} Herein, MSEA was polymerized by ATRP using relatively low amounts of copper (1000 or 100 ppm). The ATRP rate depends on the relative ratio of $[\text{Cu}^{\text{I}}-\text{L}]/[\text{X}-\text{Cu}^{\text{II}}-\text{L}]$, rather than the absolute amount of copper species (eq 1).⁴⁸

$$R_p = k_p [\text{M}] [\text{P}_n^*] = k_p K_{\text{ATRP}} \left(\frac{[\text{P}_n\text{X}][\text{Cu}^{\text{I}}-\text{L}][\text{M}]}{[\text{X}-\text{Cu}^{\text{II}}-\text{L}]} \right) \quad (1)$$

In the polymerization with 1000 ppm of Cu^{II} , the reducing agent was added at the beginning of the polymerization (following AGET procedure),⁴⁹ whereas in the reaction with 100 ppm of Cu^{II} , the reducing agent was added slowly over 5 h to ensure that some Cu^{II} deactivator complex was present throughout the polymerization (ARGET ATRP).¹⁸ The semilogarithmic plot for ARGET system indicated the steady concentration of active centers during the polymerizations but in AGET some deviation was observed (Figure 2a).⁵⁰

The M_n values estimated from GPC based on linear PMMA standards were slightly higher than the theoretical values, which could be due to either incomplete initiation or the difference in hydrodynamic volume between the GPC calibration standards (PMMA) and polyMSEA (Figure 2b), whereas GPC traces exhibited a symmetrical narrow Gaussian distribution throughout the polymerization (Figure 2c). M_n values were also calculated from ^1H NMR for each kinetic point, according to eq 2, where $\delta_{5.99}$ represents the integration area of one of the vinyl hydrogen atoms, $\delta_{4.52}$ represents the integration area of methylene peaks next to the ester group in monomer and polymer, and $\delta_{1.16}$ represents the integration area of methyl groups of EBiB. (Figure 3a). M_{MSEA} is the molar mass of monomer MSEA, and M_{EBiB} is the molar mass of initiator EBiB.

$$M_{n,\text{NMR}} = \frac{3 \times (\frac{\delta_{4.52}}{2} - \delta_{5.99})}{\delta_{1.16}} \times M_{\text{MSEA}} + M_{\text{EBiB}} \quad (2)$$

The good agreement between the theoretical values and the $M_{n,NMR}$ in Figure 2b indicated efficient initiation. In Figure 3b, the chain-end functionality was observed for an isolated polyMSEA sample prepared by AGET ATRP with 1000 ppm of Cu^{II} (sample was collected at 35% conversion with $M_{n,NMR}$ of 4000 and M_w/M_n of 1.20). Peak **a** exhibited a triplet shape associated with a quartet-like **c**. Peak **d** splitted into doublet-like shape due to chirality of the sulfoxide groups and polymer tacticity. The ratio of integration areas of **a/d** (1.00/0.27) was close to 3/1, indicating a good preservation of the chain-end functionality. The dispersity of the polymer prepared with 1000 ppm of Cu^{II} reaction remained well below 1.2 during the polymerization, while the dispersity of the polymer prepared with 100 ppm of Cu^{II} reaction was higher, 1.25 at 13.7% conversion, 1.32 at 34.4% conversion, and 1.34 at 43.1% conversion (Figure 2b). The increased dispersity can be attributed to a lower amount of deactivator (Cu^{II}). As shown in eq 3, a decrease of $[X-Cu^{II}-L]$ can increase the value of M_w/M_n .

$$\frac{M_w}{M_n} = 1 + \frac{1}{DP_n} + \left(\frac{k_p[P_nX]}{k_{deact}[X-Cu^{II}-L]} \right) \left(\frac{2}{p} - 1 \right) \quad (3)$$

Synthesis of Star Polymer

Two types of sulfoxide-containing star polymers (polyMSEA)_n-polyDVB and (poly-MSEA)_n-(β-CD) were synthesized by “arm-first” approach and “core-first” approach, respectively. The synthetic routes are shown in Scheme 2.

In the “arm-first” approach for the synthesis of (poly-MSEA)_n-polyDVB, the polyMTEA macromonomer was synthesized by ARGET ATRP in acetone, according to previous work.³¹ The kinetic plots are shown in Figure S1. A polyMTEA polymer with $M_n = 2900$ and dispersity of 1.24 was used as the macroinitiator for the synthesis of a sulfide-containing star polymer (polyMTEA)_n-polyDVB. The (poly-MTEA)_n-polyDVB was prepared by ARGET ATRP with Sn(EH)₂ as reducing agent and divinylbenzene (DVB) as cross-linker.⁵¹

As shown in Figure S2, the macroinitiator peak gradually decreased over time as the polymerization proceeded, and a new peak at higher molecular weight gradually formed and shifted to region of higher molecular weights. The reaction was stopped at conversion 91.4% and the star polymer was purified by precipitation and dialysis. The purified (polyMTEA)_n-polyDVB star polymer showed a unimodal peak with a molecular weight of $M_n = 10\ 200$ according to calibration with linear PMMA standards and $M_n = 30\ 500$ as measured by static light scattering method (Figure 4).

The (polyMSEA)_n-polyDVB star polymer was synthesized by postoxidation of the (polyMTEA)_n-polyDVB star with hydrogen peroxide solution and purified by dialysis. The oxidation was complete and no further oxidation to sulfone occurred as shown in the ¹H NMR spectra (Figure S3).⁴⁵ The (polyMSEA)_n-polyDVB had $M_{n, GPC} = 15\ 450$ and $M_{n, LS} = 33\ 450$ and narrow molecular weight distribution. The GPC values, based on the hydrodynamic volume, are smaller due to a compact structure of star-like polymers. Thus,

static light scattering method provides more accurate values. Postoxidation approach was used here due to better initiating efficiency of polyMTEA macroinitiator than polyMSEA macroinitiator.

Polymer used for biomedical applications, such as a drug delivery carrier, should be biodegradable to ensure renal clearance after delivery. β -Cyclodextrin (β -CD) is a highly water-soluble biocompatible biodegradable multifunctional molecule.⁵² Moreover, the hydrophobic cavity of cyclodextrin and its unique host–guest effect make it a good platform for hydrophobic drug delivery.⁵³ In order to prepare a star polymer with β -CD as a core, a β -CD ATRP macroinitiator with 14 attached bromoisobutyrate sites was synthesized (Figure S4)⁴⁴ by esterification of accessible hydroxyl groups. MSEA was grafted directly from the macroinitiator in a “core-first” manner by ARGET ATRP. The $M_{n, GPC} = 29\,240$ ($M_w/M_n = 1.06$) of final polymer (polyMSEA)_{IT}(β -CD) at conversion of 38.5% was measured by GPC with PMMA standards and $M_{n, LS} = 46\,300$ by static light scattering method ($M_w/M_n = 1.09$). (Figures 5 and 6).

As shown in Figure S5, the β -CD macroinitiator could be degraded in 5 wt % NaOH aqueous solution (10 mg/mL, overnight). After degradation, molecular weight of sample at ~3000 shifted to oligomer peaks at ~270. To demonstrate the biodegradability of the as-synthesized star polymer (poly-MSEA)_{IT}(β -CD), a polymer was prepared under the same conditions with conversion of 26.8%, $M_{n, GPC} = 23\,800$, and $M_w/M_n = 1.05$. The sample was treated with 5 wt % NaOH aqueous solution overnight (10 mg/mL). After degradation, the original star peak shifted to $M_{n, GPC} = 2300$ with dispersity $M_w/M_n = 1.03$, a size small enough for removal from the bloodstream (Figure 7).⁵⁴ Therefore, such a high molecular weight biodegradable star-like structure, with low molecular weight side-arms, can be tested for drug delivery applications, especially for delivery of small hydrophobic drugs.⁵³

The glass transition temperature (T_g) was investigated by differential scanning calorimetry (DSC). The (polyMSEA)_{IT}-polyDVB with $M_n = 33\,450$ ($M_w/M_n = 1.59$) had a $T_g = 31.5\text{ }^\circ\text{C}$ while a linear polyMSEA with $M_n = 5200$ ($M_w/M_n = 1.18$) had $T_g = 15.7\text{ }^\circ\text{C}$ (Figure S6).

Water-Solubility and Biocompatibility

Many biocompatible polymers, such as PEG, POZ, polyacrylamides, and polyOEGMA exhibit lower critical solution temperature (LCST) behavior. The LCST could be advantageous for drug delivery, but it may decrease solubility in water, especially at elevated temperature. More hydrophobic polymer can be attached to opsonins, leading to early bloodstream removal. Therefore, to evaluate the water-solubility and hydrophobicity of polyMSEA, its solution behavior was studied at different temperatures. Dynamic light scattering (DLS) was used in the temperature range of 25–92 °C. Since DLS analysis of pure polyMSEA showed no aggregation within the investigated temperature range (Figure 8a), the LCST of copolymers with variable composition were used and the LCST of polyMSEA was estimated via extrapolation to pure polyMSEA. Thus, free radical copolymerization of NIPAM and MSEA was carried out at different molar ratios of comonomers. DLS traces for polyMSEA-co-polyNIPAM copolymers with different fractions of MSEA are presented in Figures S7–S9.

As shown in Figure 8b, as the fraction of MSEA increased, the LCST of the copolymer also increased. By extrapolating the observed LCST of the copolymers to a composition of 100% polyMSEA, its LCST was estimated to be ~140 °C. This suggests that a neutral water-soluble polyMSEA should not have solubility limitations when utilized in biorelated applications. To evaluate direct solubility, 359 mg of polyMSEA with $M_n = 14\,400$ was fully dissolved in 1 mL deionized water at room temperature, which compares to the solubility of NaCl in water at the same temperature (Figure S10).

In addition, reactivity ratios of MSEA (monomer 1) vs NIPAM (monomer 2) were determined by Kelen-Tüdös method: $r_1 = 1.24$ and $r_2 = 0.42^{42,43}$ (Figures S11 and S12). Therefore, the sample prepared for LCST extrapolation is not pure random copolymers and can have some composition drift. The reactivity ratio of MSEA in copolymerization with three other acrylic monomers, methyl acrylate, *n*-butyl acrylate, and oligo(ethylene glycol) methyl ether acrylate (OEGA₄₈₀), were also calculated by the Kelen-Tüdös method with values of r_1/r_2 as 0.49/1.01, 0.49/0.83, and 0.77/0.33, respectively (Figures S11 and S12).

Finally, a cytotoxicity, of polyMSEA was examined by cell viability assessments using human embryonic kidney cells (HEK 293).⁵⁵ HEK 293 cells were grown in a 96 well tissue culture plate at a density of 10 000 cells/well for 24 h. The monomer MSEA, linear polyMSEA ($M_n = 14\,400$, $M_w/M_n = 1.40$), and star polymer (polyMSEA)_{*n*}-polyDVB ($M_n = 33\,450$, $M_w/M_n = 1.59$) were dissolved in PBS at a concentration of 100 mg/mL and added to the tissue culture plate at different amounts. After 48 h, ATP Cell Titer Glo Assay was used and luminescence was measured and quantified as a percentage of the control wells. As shown in Figure 9, both linear polyMSEA and (polyMSEA)_{*n*}-polyDVB star showed no toxicity, even at concentration up to 3 mg/mL in comparison to the control group (only cell without addition of other chemicals). This result proved that polymeric analogues of DMSO prepared by ATRP possess low cytotoxicity, similar to their monomeric counterpart: DMSO. However, the cell viability decreased by 90% for the monomer MSEA at concentration of only 0.1 mg/mL. Such relatively high toxicity of MSEA is anticipated due to the presence of reactive acrylate groups that can react with amine groups or thiol groups in the cell environment.⁵⁶

In future work, the polymer will be evaluated for preparation of therapeutic protein-polymer conjugates, use in gene knockdown therapy, and other applications requiring water-soluble biocompatible polymeric materials. The immunogenicity of the polyMSEA-based biohybrids will also be examined.

CONCLUSIONS

In conclusion, the well-defined biocompatible polymeric analogues of DMSO, based on a sulfoxide-containing monomer 2-(methylsulfinyl)ethyl acrylate (MSEA), were prepared by ATRP. Well-defined star polymers were synthesized by two approaches, an “arm-first” approach with DVB as cross-linker and a “core-first” approach with a biodegradable β -cyclodextrin core. LCST of polyMSEA ca. 140 °C was estimated from NIPAM copolymers by extrapolation to pure polyMSEA. Both linear polyMSEAs and star polymers, (polyMSEA)_{*n*}-polyDVB, showed no cytotoxicity toward human embryonic kidney cells

(HEK 293). Thus, the polymeric analogue of DMSO has the following advantages: (1) straightforward synthesis from commercially available starting materials, (2) small steric hindrance, high water-solubility and low cytotoxicity, and (3) potential conjugation by grafting-onto or grafting-from (bio)-functional compounds/substrates via ATRP. The water-soluble and nontoxic polymeric analogue of DMSO could be used in some biomedical applications and/or potentially replace such polymers as PEG, polyOEGMA, and polyoxazolines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
 ^1H NMR spectrum of MSEA.

Figure 2.

(a) Kinetic plots of $\ln([M]_0/[M])$ vs time, (b) plots of M_n and M_w/M_n vs conversion and (c) GPC trace for ATRP of MSEA. AGET conditions: $[\text{MSEA}]_0/[\text{EBiB}]_0/[\text{CuBr}_2]_0/[\text{TPMA}]_0/[\text{AA}]_0 = 70/1/0.07/0.15/0.7$, 35 °C. ARGET conditions: $[\text{MSEA}]_0/[\text{EBiB}]_0/[\text{CuCl}_2]_0/[\text{TPMA}]_0/[\text{AA}]_0 = 70/1/0.007/0.015/0.14$ (AA added over 5 h), 35 °C. (AA: ascorbic acid)

Figure 3.

Example of ^1H NMR spectrum for calculation of $M_{n,\text{NMR}}$. (Samples collected during polymerization by syringe and diluted in $\text{DMSO}-d_6$) (a) and ^1H NMR spectrum of an isolated polyMSEA with a preserved chain-end functionality (b).

Figure 4. dRI GPC traces of macroinitiator polyMTEA, (poly-MTEA)_n-polyDVB, and (polyMSEA)_n-polyDVB (a) and static light scattering traces of (polyMTEA)_n-polyDVB and (polyMSEA)_n-polyDVB (b).

Figure 5. Synthesis of star polymer with β -cyclodextrin core. Kinetic plot of $\ln([M]_0/[M])$ vs time (a), plots of M_n and M_w/M_n vs conversion (b). (Red curve: theoretical M_n , black square: M_n by GPC, open square: M_w/M_n by GPC)

Figure 6. GPC traces for ARGET ATRP of MSEA from β -CD macroinitiator (a) and static light scattering trace of the same sample at conversion of 38.5% (b).

Figure 7. GPC traces of (polyMSEA)_n-(β -CD) before and after degradation by 5 wt % NaOH aqueous solution.

Figure 8. DLS data of polyMSEA in water from 25 to 92 °C (a) and LCST of polyMSEA-co-polyNIPAM copolymers with different compositions (red curve: linear fitting with extrapolation to 100% polyMSEA) (b).

Figure 9.

HEK 293 Cell toxicity of linear polyMSEA ($M_n = 14\,400$, $M_w/M_n = 1.40$), star polymer (polyMSEA)_n-polyDVB ($M_n = 33\,450$, $M_w/M_n = 1.59$) and monomer MSEA.

Scheme 1.
Synthesis of MTEA and MSEA Monomers

Scheme 2.

Synthesis of Star Polymer (polyMSEA)_n-polyDVB by “Arm-First” Approach and (polyMSEA)_n-(β-CD) by “Core-First” Approach