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Oligoethylene-Glycol-Functionalized Polyoxythiophenes for Cell Engineering: Syntheses, Characterizations, and Cell Compatibilities

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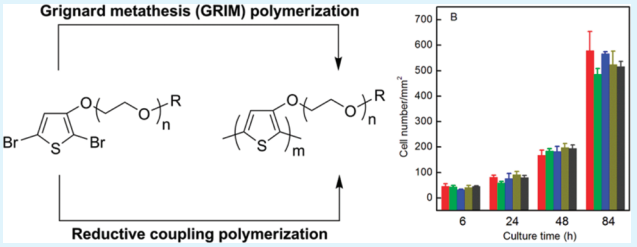
Supporting Information

ABSTRACT: A series of methyl- or benzyl-capped oligoethylene glycol functionalized 2,5-dibromo-3-oxythiophenes are synthesized and successfully polymerized by either Grignard metathesis (GRIM) polymerization or reductive coupling polymerization to yield the corresponding polymers in reasonable yields and molecular weights with narrow molecular weight distribution. These synthesized polyoxythiophenes exhibit high electroactivity and stability in aqueous solution when a potential is applied. Polyoxythiophenes from different polymerization approaches display different colors after purification and spectroelectrochemical studies confirm that the difference of color is from the difference of doping state. Little cytotoxicity is observed for the polymers by in vitro cell compatibility assay. NIH3T3 fibroblast cells are well attached and proliferate on spin-coated films. These results indicate that oligoethylene-glycol-functionalized polyoxythiophenes are promising candidates as conducting biomatierals for biomedical and bioengineering applications.



KEYWORDS: *conducting polymers, conjugated materials, bioengineering, polymerization, cross-coupling reaction, biomaterials, oligoethylene glycol*

INTRODUCTION

Recent advances in biomedical engineering show that electrical stimulations are capable of regulating a variety of cellular activities, including DNA synthesis, cell adhesion, migration, proliferation, and differentiation.[1,2] In the search for materials capable of meeting both the electrical and the bioengineering requirements of the interface, electrical conducting polymers (ECPs) are attractive candidates because of their softer nature (compared to metals and silicon), diversified molecular architecture, controlled electronic and ionic charge transport properties, and ability to deliver electrical signals locally. After Langer group[3,4] and Wallace group[5] pioneered the research on interfacing cells and ECPs, considerable research efforts have been carried out on the development of organic conductive biomaterials, including polypyrrole (PPy),[5−8] polyaniline (PAni),[9−14] polythiophene,[15,16] and poly(3,4-ethylenedioxythiophene) (PEDOT),[17−20] as cell engineering scaffolds or substrates. Unfortunately, the applications toward biomedical devices of these polymers are limited due to the absence of a cell interaction site, hydrophobicity of the polymers, or poor solubility for engineering process. Therefore, the development of conducting polymers that are capable of intimate and noninvasive integration with cells and soft biological tissues still remains challenging.

The synthesis of soluble, especially water-soluble, conductive biomaterials becomes a promising research focus because it allows the investigation of important features required for bioengineering, including biodegradability, hydrophilicity−hydrophobicity switch, facile process, and incorporation with biomacromolecules, to integrate with electrical stimulation. In an earlier report, Langer and co-workers prepared PPy-based bioerodible conducting polymers containing ionizable or hydrolyzable side groups.[21] Recently, the Wallace group reported an erodible film by layer-by-layer assembly of anionic sulfonated polythiophene and cationic poly(ethyleneimine).[22] The multilayered film was electroactive, biodegradable, and promoted cell adhesion and growth. Wei and co-workers incorporated aniline pentamer into polylactide.[23,24] The obtained triblock or multiblock copolymers could accelerate the differentiation of PC-12 cells. Similar approaches were also applied to oligopyrroles and oligothiophene to obtain a degradable and biocompatible conductive biomaterial.[25] Oligoaniline was also incorporated with polysaccharides to form a conductive polysaccharide hydrogel which combined the film formation properties, biocompatibility, and biodegradability of polysaccharides and the electroactivity of oligoanilines.[26] Electrically conductive hydrogel composites consisting of oligo(polyethylene glycol) fumarate and PPy were developed by Yaszemski and co-workers for applications in nerve regeneration.[27] Although many systems have been developed and studied, one common concern of PAni, PPy, and polyalkylthiophenes for biomedical applications is their stabilities in the biological environments when a potential is applied.[28,29]

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Unlike PPy, PAni and polyalkythiophene, water dispersible, and commercially available poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT-PSS) started to draw attention quite recently. It is mainly due to the more desirable properties, including reduced band gap, low oxidation potential, and highly stable conducting state.[30] However, PEDOT-PSS contains a nonconductive component, PSS, and it can be processed only as a colloidal suspension, which limits its biomedical applications. After surveying the literature, it is obvious that conducting polymers with long-term stabilities in biological environments and facile processablility are still a challenge. Introduction of ethylene glycol group usually increases the aqueous stability of the polymer and be able to control the protein and cell binding on the material interface. The neutral nature of ethylene glycol is also insensitive toward environmental pH change and is generally less toxic to cells. Herein, we report the synthesis of oligoethylene glycol (OEG) functionalized polyoxythiophenes, a new class of conducting polymer, by transition metal catalyzed polymerization approach. The transition-metal-catalyzed polymerization often yields polymers with high molecular weight, narrow molecular weight distribution, and good regioregularity.[31,32] We also investigate the polymers' electrochemical and spectroelectrochemical properties. Furthermore, cell compatibility of these polymers is also examined. We anticipate that the OEG side chains of conjugated polyoxythiophene would increase the solubility in aqueous solution, and biocompatibility, and electrochemical stability, resulting in promising conductive biomaterials.

EXPERIMENTAL SECTION

General Methods. Nuclear magnetic resonance (NMR) spectra were obtained at 25 °C on a Varian 500 spectrometer. Chemical shifts were referenced to residual solvent. The polymer molecular weights and the molecular weight distributions were determined at 40 °C by gel permeation chromatography (GPC) on a HLC-8220 GPC apparatus (Tosoh Corp.) with THF as an eluent at a flow rate of 0.35 mL/min. The molecular weight was calibrated by polystyrene standard. UV−vis spectra were measured on a Jasco V-630 spectrophotometer. Mass spectra were recorded on JMS-700 V (JEOL). All electrochemical measurements were performed with an Autolab PGSTAT 128N potentiostat (Metrohm). Cyclic voltammetry was performed in a three-electrode cell versus a quasi-internal Ag wire reference electrode submersed in 0.01 M $AgNO_3$/0.1 M nBu$_4$NPF$_6$ in CH_3CN. Typical cyclic voltammograms were recorded by using indium tin oxide (ITO) coated glass slides as the working electrode and a platinum coil counter electrode. Contact angle measurements were performed on a SImage mini contact angle measuring instrument (Excimer). The film surface morphology was examined using atomic force microscopy (NanoScope V, Vecco) with silicon cantilevers (Pointprobe NCH probes, NanoWorld).

Materials. Unless indicated, all reagents and solvents were obtained from commercial suppliers (Sigma-Aldrich, Wako Chemicals, TCI) and were used without further purification. 2-(2-hydroxyethoxy)ethoxy)thiophene (TH-EG2-OH) and (2-(2-(2-(2-hydroxyethoxyl)ethoxy)ethoxy) ethoxy)thiophene (TH-EG4-OH) were prepared as reported.[33,34]

3-(2-(2-Methoxyethoxy)ethoxy)thiophene (TH-EG2-OMe). In a dry 100 mL three-neck round-bottom flask containing TH-EG2-OH (5.6 g, 30 mmol) and anhydrous THF (100 mL), NaH (1.2 g, 50 mmol) was added slowly under N_2. The solution was stirred for 1 h and iodomethane (7.0 g, 50 mmol) was then added. The reaction mixture was stirred under N_2 overnight. After the reaction was completed, the mixture was poured into a 1 M NH_4Cl aqueous solution (100 mL) and extracted with ethyl acetate for several times. The combined organic layer was washed with saturated NaCl solution and dried with anhydrous $MgSO_4$. The solvent was then removed under reduced pressure. The crude compound was further purified by column chromatography (ethyl acetate) on silica gel to yield colorless liquids (5.9 g, 97%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.39 (s, 3H), 3.57 (m, 2H), 3.71 (m, 2H), 3.84 (m, 2H), 4.12 (m, 2H), 6.25 (dd, 1H, J = 3.0, 1.5 Hz), 6.77 (dd, 1H, J = 5.5, 1.5 Hz), 7.16 (dd, 1H, J = 5.5, 3.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 59.1, 69.5, 69.7, 70.7, 71.9, 97.5, 119.6, 124.6, 157.6. HR-MS (EI) calculated for $C_9H_{14}O_3S$ 202.0664 [M^+]; found 202.0674.

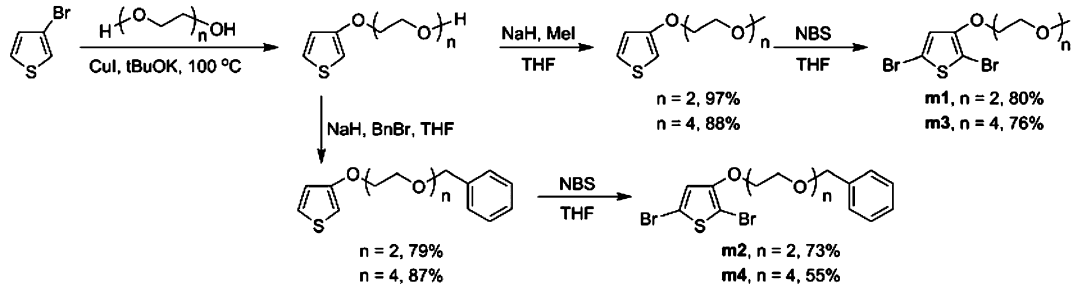
3-(2-(2-Benzyloxyethoxy)ethoxy)thiophene (TH-EG2-OBn). In a dry 100 mL three-neck round-bottom flask containing TH-EG2-OH (5.6 g, 30 mmol) and anhydrous THF (100 mL), NaH (1.2 g, 50 mmol) was added slowly under N_2. The solution was stirred for 1 h and benzyl bromide (8.5 g, 50 mmol) was then added. The reaction mixture was stirred under N_2 overnight. After the reaction was completed, the mixture was poured into a 1 M NH_4Cl aqueous solution (100 mL) and extracted with ethyl acetate for several times. The combined organic layer was washed with saturated NaCl solution and dried with anhydrous $MgSO_4$. The solvent was then removed under reduced pressure. The crude compound was further purified by column chromatography (ethyl acetate) on silica gel to yield colorless liquids (6.6 g, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.66 (m, 2H), 3.74 (m, 2H), 3.85 (m, 2H), 4.12 (m, 2H), 4.57 (s, 2H), 6.26 (dd, 1H, J = 3.0, 1.5 Hz), 6.78 (dd, 1H, J = 5.5, 1.5 Hz), 7.16 (dd, 1H, J = 5.5, 3.0 Hz), 7.33 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 69.5, 69.6, 69.7, 70.9, 73.3, 97.5, 119.6, 124.6, 127.6, 127.7, 128.4, 138.2, 157.6. HR-MS (EI) calculated for $C_{15}H_{18}O_3S$ 278.0977 [M^+]; found 278.0968.

3-(2-(2-(2-(2-Methoxyethoxyl)ethoxy)ethoxy)ethoxy)-thiophene (TH-EG4-OMe). In a dry 100 mL three-neck round-bottom flask containing TH-EG4-OH (8.3 g, 30 mmol) and anhydrous THF (100 mL), NaH (1.2 g, 50 mmol) was added slowly under N_2. The solution was stirred for 1 h and iodomethane (7.0 g, 50 mmol) was then added. The reaction mixture was stirred under N_2 overnight. After the reaction was completed, the mixture was poured into a 1 M NH_4Cl aqueous solution (100 mL) and extracted with ethyl acetate for several times. The combined organic layer was washed with saturated NaCl solution and dried with anhydrous $MgSO_4$. The solvent was then removed under reduced pressure. The crude compound was further purified by column chromatography (ethyl acetate) on silica gel to yield colorless liquids (7.6 g, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.37 (s, 3H), 3.53 (t, 2H, J = 4.0 Hz), 3.64−3.71 (m, 10H), 3.84 (t, 2H, J = 4.0 Hz), 4.10 (t, 2H, J = 5.0 Hz), 6.25 (dd, 1H, J = 3.0, 1.5 Hz), 6.76 (dd, 1H, J = 5.5, 1.5 Hz), 7.15 (dd, 1H, J = 5.5, 3.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 59.1, 69.6, 69.7, 70.5, 70.6, 70.6, 70.6, 70.8, 71.9, 97.5, 119.6, 124.6, 157.6. HR-MS (EI) calcd for $C_{13}H_{22}O_5S$ 290.1188 [M^+]; found 290.1195.

Synthesis of 3-(2-(2-(2-(2-Benzyloxyethoxyl)ethoxy)ethoxy)-ethoxy)thiophene (TH-EG4-OBn). In a dry 100 mL three-neck round-bottom flask containing TH-EG4-OH (8.3 g, 30 mmol) and anhydrous THF (100 mL), NaH (1.2 g, 50 mmol) was added slowly under N_2. The solution was stirred for 1 h and benzyl bromide (8.5 g, 50 mmol) was then added. The reaction mixture was stirred under N_2 overnight. After the reaction was completed, the mixture was poured into a 1 M NH_4Cl aqueous solution (100 mL) and extracted with ethyl acetate for several times. The combined organic layer was washed with saturated NaCl solution and dried with anhydrous $MgSO_4$. The solvent was then removed under reduced pressure. The crude compound was further purified by column chromatography (ethyl acetate) on silica gel to yield colorless liquids 9.5 g, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.61−3.83 (m, 12H), 3.82 (t, 2H, J = 5.0 Hz), 4.10 (t, 2H, J = 5.0 Hz), 4.56 (s, 2H), 6.24 (dd, 1H, J = 3.0, 1.5 Hz), 6.76 (dd, 1H, J = 5.5, 1.5 Hz), 7.15 (dd, 1H, J = 5.5, 3.0 Hz), 7.32 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 69.4, 69.6, 69.7, 70.6, 70.6, 70.6, 70.6, 70.8, 73.2, 97.5, 119.6, 124.6, 127.6, 127.7,128.3, 138.3, 157.6. HR-MS (EI) calcd for $C_{19}H_{26}O_3S$ 366.1501 [M^+]; found 366.1510.

2,5-Dibromo-3-(2-(2-methoxyethoxy)ethoxy)thiophene (m1). In a dry 100 mL round-bottom flask containing TH-EG2-OMe (3.04 g, 15 mmol) and dry THF (100 mL) under N_2, N-bromosuccinimide (NBS) (5.84 g, 33 mmol) was added under 0

Scheme 1. Syntheses of Oligoethylene-Glycol-Functionalized Thiophene Monomers



°C. After the reaction mixture was stirred for 1 h at room temperature, water (100 mL) was added. The reaction mixture was then extracted with ethyl acetate (100 mL). The combined organic layer was washed with saturated NaCl solution and dried with anhydrous $MgSO_4$. The solvent was then removed under reduced pressure. The crude compound was further purified by column chromatography (ethyl acetate) on silica gel to yield light yellow liquid (4.3 g, 80%). The compound needed to be stored in freezer without exposing to light. δ ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.39 (s, 3H), 3.56 (t, 2H, J = 4.5 Hz), 3.70 (t, 2H, J = 4.5 Hz), 3.79 (t, 2H, J = 5.0 Hz), 4.16 (t, 2H, J = 5.0 Hz), 6.81 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 59.1, 69.8, 70.9, 72.0, 72.0, 91.4, 109.6, 121.5, 153.7. HR-MS (EI) calcd for $C_9H_{12}Br_2O_3S$ 357.8874 [M^+]; found 357.8864.

2,5-Dibromo-3-(2-(2-benzyloxyethoxy)ethoxy)thiophene (m2). Using a similar procedure for the synthesis of **m1**, TH-EG2-OBn was reacted with NBS to yield light yellow liquid (4.6 g, 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.64 (t, 2H, J = 4.5 Hz), 3.73 (t, 2H, J = 4.5 Hz), 3.80 (t, 2H, J = 5.0 Hz), 4.16 (t, 2H, J = 5.0 Hz), 4.57 (s, 2H), 6.81 (s, 1H), 7.34 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 69.5, 69.8, 71.0, 72.1, 73.3, 91.4, 109,6, 121.5, 127.6, 127.7, 128.4, 138.2, 153.7. HR-MS (EI) calcd for $C_{15}H_{16}Br_2O_3S$ 435.9187 [M^+]; found 435.9198.

2,5-Dibromo-3-(2-(2-(2-(2-methoxyethoxyl)ethoxy)ethoxy)-ethoxy)thiophene (m3). Using a similar procedure for the synthesis of **m1**, TH-EG4-OMe was reacted with NBS to yield light yellow liquid (8.8 g, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.37 (s, 3H), 3.53 (t, 2H, J = 6.0 Hz), 3.63−3.71 (m, 10 H), 3.78 (t, 2H, J = 5.0 Hz), 4.15 (t, 2H, J = 5.0 Hz), 6.80 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 59.0, 69.8, 70.5, 70.6, 70.6, 70.6, 71.0, 71.9, 72.0, 91.4, 109.6, 121.4, 153.7. HR-MS (EI) calcd for $C_{13}H_{20}Br_2O_5S$ 445.9398 [M^+]; found 445.9391.

2,5-Dibromo-3-(2-(2-(2-(2-benzyloxyethoxyl)ethoxy)-ethoxy)ethoxy)thiophene (m4). Using a similar procedure for the synthesis of **m1**, TH-EG4-OBn was reacted with NBS to yield light yellow liquid (8.6 g, 55%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.61 −3.71 (m, 12H), 3.78 (t, 2H, J = 5.0 Hz), 4.13 (t, 2H, J = 5.0 Hz), 4.56 (s, 2H), 6.80 (s, 1H), 7.32 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 69.4, 69.8, 70.6, 70.7, 70.7, 70.7, 71.0, 72.0, 73.2, 91.3, 109.6, 121.4, 127.6, 127.7, 128.3, 138.2, 153.7. HR-MS (EI) calcd for $C_{19}H_{24}Br_2O_5S$ 521.9711 [M^+]; found 521.9720.

General Procedure for Preparation of Polyoxythiophenes from 2,5-Dibromo-3-oxythiophene by the Grignard Metathesis Polymerization (GRIM). To a dry glass tube with a three-way stopcock side arm containing 2,5-dibromo-3-(2-(2-methoxyethoxy)-ethoxy)thiophene (**m1**) (720 mg, 2 mmol) in nitrogen was added anhydrous THF (10 mL) and isopropylmagnesium chloride (1 mL, 2.0 M in THF). After the reaction mixture was stirred for 1 h at ambient temperature, Ni(dppp)Cl_2 (55 mg, 0.1 mmol) was added. The reaction mixture was stirred for an additional hour and then quenched by adding 1 M HCl (1 mL). The crude mixture was then poured into a mixture of MeOH (100 mL) and H_2O (100 mL). The polymer was then filtered and dried under vacuum to yield dark blue powder (265 mg, 66%).

General Procedure for Preparation of Polyoxythiophene from Reductive Coupling Polymerization of 2,5-Dibromo-3-oxythiophene. To a dry glass tube with a three-way stopcock side arm were added zinc (195 mg, 6 mmol) and $NiCl_2$ (130 mg, 0.5 mmol), and the mixture was heat-dried under reduced pressure. After the glass tube was cooled to room temperature in nitrogen, triphenylphosphine (512 mg, 2 mmol), **m1** (720 g, 2 mmol), and dry DMF (2 mL) were added to the glass tube in the glovebox. The reaction mixture was stirred at 90 °C for 48 h and then poured into a mixture of MeOH (100 mL) and H_2O (100 mL). Residual impurities were removed by redissolution in THF and precipitation in MeOH/H_2O for several times. The polymer was eventually dried under vacuum to yield dark red powders (442 mg, 98%).

In vitro Cytotoxicity Test. Polymer cytotoxicity was examined by a MTT assay which assessed the mitochondrial activity in living cells. Poly(**1**−**4**) were spincasted onto glass slides and sterilized for 15 min in 70% ethanol. The controlled experiments were conducted on glass slides without polymer coatings. NIH3T3 fibroblast cells (3×10^4 cells/well) were seeded in Dulbecco's modified eagle medium (DMEM) with 10% bovine serum. After cell seeding for 72 h, MTT assay was performed to determine cellular metabolic activity by spectral absorbance. For every test, the data are expressed as means the standard deviation (n = 3). NIH3T3 fibroblasts were also used as the model to evaluate the cell attachment and proliferation. Polyoxythiophene thin films was prepared by spin coating on a glass slide (1 cm × 1 cm) and were UV sterilized before being used for cell studies. The slides were placed is tissue culture plate and NIH3T3 fibroblasts were seeded at a density of 2×10^4/mL cells on each sample in the DMEM with 10% bovine serum and incubated at 37 °C with 5% CO_2. Attached cells were observed with a phase contrast microscope after 6, 24, 48, and 84 h respectively. For every sample, the data are expressed as means the standard deviation (n = 3). The control measurements were performed on tissue culture plate without any glass slides or coatings.

RESULT AND DISCUSSION

Syntheses of Monomers and Polymers. Recent advances in transition-metal-catalyzed cross-coupling reactions enable researchers to prepare well-defined and regioregular conjugated polymers by step reaction polymerization with narrow polymer molecular weight distribution. Conventionally, substituted conductive polythiophenes can be synthesized with a variety of cross coupling reactions,[35] including Grignard metathesis (GRIM) polymerization, Suzuki coupling reaction, Stille coupling reaction, and reductive coupling polymerization. In the case of Suzuki and Stille coupling reactions, it is necessary to synthesize the coupling partners as boric or tin containing thiophene monomers. On the other hand, GRIM and reductive coupling polymerizations are more simple and direct because these two approaches allow the polymerization to be carried out with dibrominated thiophene monomers.[31,32] Therefore, our research focuses on the syntheses of a series of oligoethylene glycol (OEG)-containing 2,5-dibromothiophene monomers. These monomers are then polymerized using GRIM and reductive coupling polymerization.

Methyl or benzyl capped diethylene glycol (EG2) and tetraethylene glycol (EG4) containing dibromothiophene

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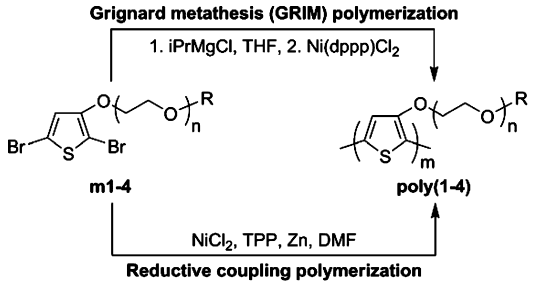
monomers were synthesized in reasonable yield as shown in Scheme 1. 3-Bromothiophene was first reacted with oligo-ethylene glycol to form OEG-substituted oxythiophenes. After the oxythiophenes were methylated or benzylated, they underwent dibromination to yield **m1−4** in good yields (55−80%). The molecular structures of the monomers were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance spectra and mass measurements. Monomers **m1−4** were then subjected to both GRIM[36] and reductive coupling polymerization[37] as shown in Scheme 2. In GRIM polymerization, the

Scheme 2. Approaches for Polymerization



monomers were first reacted with a Grignard reagent, iPrMgCl, and a mixture of regio-isomers of thiophene-magnesium intermediates was obtained. The step reaction polymerization was initiated by the subsequent addition of Ni(dppp)Cl_2 catalyst. The polymerization was conducted in tetrahydrofuran at room temperature for 1 h. Reductive coupling polymerizations were conducted in one pot using DMF as solvent at a elevated temperature (90 °C) for 48 h. In the reaction mixture, $NiCl_2$, zinc metal and triphenylphosphine (TPP) were also added. TPP was excessive to $NiCl_2$ in order to suppress the possible side reactions.

The polymerization results were summarized in Table 1. The polymerization of **m1** and **m2** by GRIM method gave the

Table 1. Polymerization of m1−m4 by Grignard Metathesis Polymerization[a] and Reductive Coupling Polymerization[b]

monomer	catalyst	polymer	yield (%)	Mn[c]	M_w/M_n[c]	DP
m1	iPrMgCl, Ni(dppp)Cl_2	poly(**1**)-Mg, dark blue	66	2800	1.2	14
m2	iPrMgCl, Ni(dppp)Cl_2	Poly(**2**)-Mg, dark blue	48	4800	1.3	17
m1	$NiCl_2$, TPP, Zn	poly(**1**)-Zn, dark red	98	2200	1.1	11
m2	$NiCl_2$, TPP, Zn	poly(**2**)-Zn, dark red	79	2200	1.1	8
m3	$NiCl_2$, TPP, Zn	poly(**3**)-Zn, ferric red	29	2700	1.4	9
m4	$NiCl_2$, TPP, Zn	poly(**4**)-Zn, ferric red	48	3100	1.5	8

[a]Reaction condition: monomers are first reacted with iPrMgCl (1 equiv), and then added Ni(dppp)Cl_2 (0.03 equiv) in THF. [b]Reaction condition: $NiCl_2$ (0.25 equiv), TPP (1 equiv), and Zn (3 equiv). [c]Determined by GPC results based on polystyrene standards using THF as eluent.

corresponding polymers (poly(**1**)-Mg and poly(**2**)-Mg) in reasonable yields. However, polymerization of more hydrophilic **m3** and **m4** failed to yield solid polymers. It was most likely due to the high polarity of ethylene glycol side chain. This could also be due to the formation of metal complex by a stronger coordination of tetraethylene glycol moiety. Therefore, polymers of lower molecular weights were obtained comparing to the typical poly(3-alkylthiophene) obtained from GRIM methods. In the literature, only diethylene glycol (EG2) and triethylene glycol (EG3) functionalized polyoxythiophenes have been reported.[36] In the case of reductive coupling polymerization, all monomers **m1−4** were successfully polymerized to afford corresponding polymers poly(**1−4**)-Zn. The yields of poly(**3**)-Zn and poly(**4**)-Zn were lower than those of poly(**1**)-Zn and poly(**2**)-Zn. This could be attributed to the loss of polymer products during workups of the more hydrophilic poly(**3**)-Zn and poly(**4**)-Zn. Unfortunately, high molecular weight polymer could not be obtained although various reaction conditions were examined. On the other hand, low-molecular-weight polymer might be more appropriate for in vivo bioengineering applications because they could pass through the metabolic system more rapidly without toxic accumulation inside the body. All polymers synthesized displayed narrow molecular weight distribution and were amorphous and soluble in common organic solvents, including tetrahydrofuran, dichloromethane, chloroform, acetone, and DMF.

Spectroelectrochemical Characterization. Polymers from GRIM and reductive coupling polymerization exhibited different colors after purification as shown in Table 1 and Figure 1A and 1B. Poly(**1**)-Mg and poly(**2**)-Mg were obtained

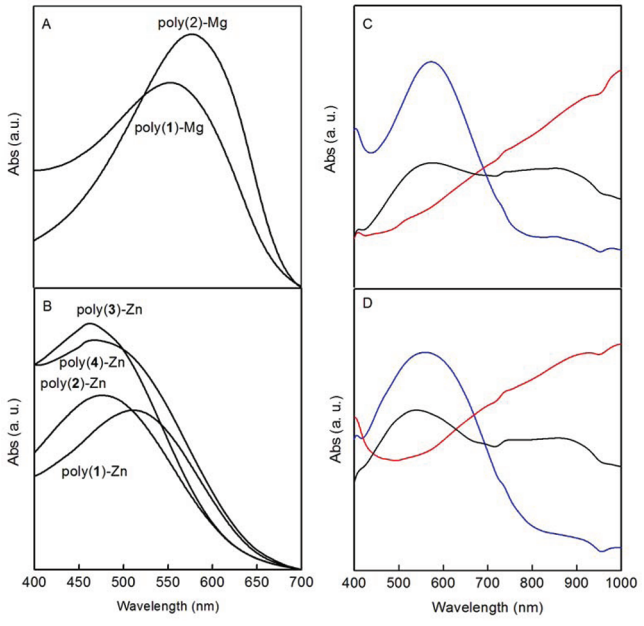


Figure 1. UV−visible spectra of poly(**1−4**): (A) from GRIM polymerization in THF (c = 0.1 mM); (B) from reductive coupling polymerization in THF (c = 0.1 mM). Spectroelectrochemical characterization of (C) poly(**1**)-Mg and (D) poly(**1**)-Zn dropcasted film on ITO-coated glass slides by applying voltages at −0.6 V (blue), 0 V (black), and 0.6 V (red) in 0.1 M $LiClO_4$ aqueous solution using a Ag/AgCl reference electrode.

in dark blue color with the maximum absorption peak at around 550−580 nm. On the other hand, poly(**1−4**)-Zn displayed dark red color and maximum absorption peak at 450−510 nm. We hypothesized that the difference of peak absorptions might be due to the doping level difference of the polymers from GRIM and reductive coupling methods. The existence of zinc metal in

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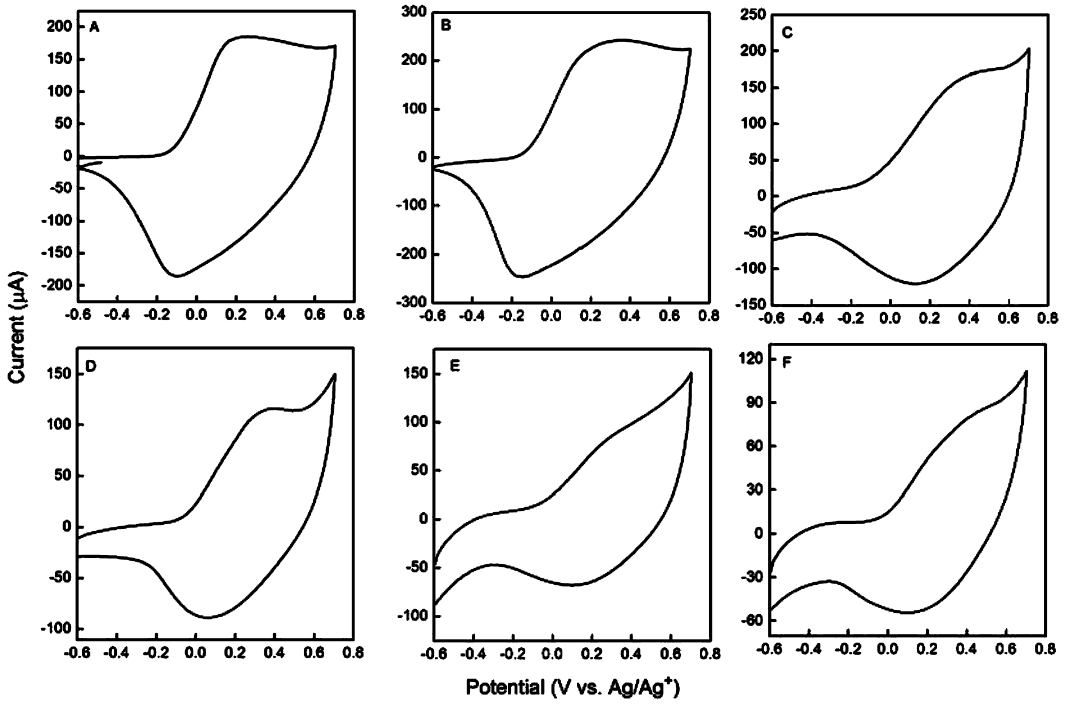

Figure 2. Cyclic voltammograms of dropcasted films of (A) poly(**1**)-Mg, (B) poly(**2**)-Mg, (C) poly(**1**)-Zn, (D) poly(**2**)-Zn, (E) poly(**3**)-Zn, and (F) poly(**4**)-Zn on gold-coated glass slides in propylene carbonate solution containing 0.1 M nBu$_4$NPF$_6$ electrolyte at a scan rate of 100 mV/s. The measurements were done using a Ag/Ag$^+$ reference electrode.

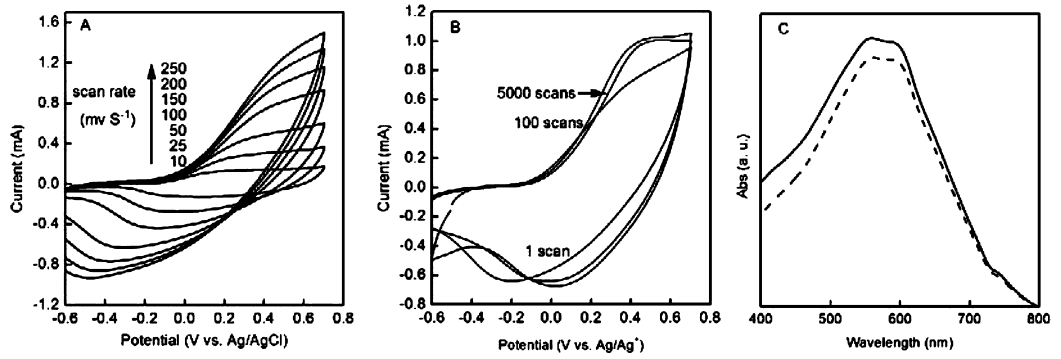

Figure 3. Electrochemical properties of polyoxythiophenes in 0.1 M LiClO$_4$ aqueous solution using a Ag/AgCl reference electrode: (A) Cyclic voltammograms of dropcasted poly(**1**)-Mg film at various scan rates, (B) Representative cyclic voltammograms of dropcasted poly(**1**)-Mg film for 5000 cycles at a scan rate of 100 mV/s, and (C) UV−visible spectra of drop-casted film incubated in PBS buffer before (solid line) and after (dashed line) 4 days.

the reductive coupling polymerization reduced the synthesized polymer to the nondoped state. Therefore, the maximum absorption peak shifted to shorter wavelength.

To confirm this hypothesis, spectroelectrochemical studies on the UV−visible spectra of polymers under various oxidation states were carried out. After the polymer films were dropcasted on indium tin oxide (ITO)-coated glass slides, the polymer-coated slides were immersed in aqueous solution containing 0.1 M LiClO4 electrolyte with a platinum counter electrode and a Ag/AgCl reference electrode. As shown in Figure 1C, D, both poly(**1**)-Mg and poly(**1**)-Zn displayed similar UV−visible absorption at fully reduced state with peak absorption at ∼550 nm when applying a potential at −0.6 V. We observed that the absorption wave of poly(**1**)-Zn was broader and this could be attributed to the different regioregularity of the polymers from different polymerization methods. It was known that GRIM polymerization yielded more region-regular (head-to-tail linked) polymers. These region-regular polymers should result in narrower peak absorption wave. When the polymers were oxidized at 0 and 0.6 V, peak absorptions were shifted to higher wavelength of near IR region gradually due to the oxidation to create radical cations and dications. Similarity in the UV−visible spectra transformations between poly(**1**)-Mg and poly(**1**)-Zn supported our hypothesis that the difference of polymer original colors were due to the variation of polymer doping level.

Electrochemical Characterization and Stability. The redox behaviors of the synthesized polymers were studied by cyclic voltammetry (CV) in propylene carbonate solution containing 0.1 M nBu$_4$NPF$_6$ electrolyte using a platinum counter electrode and a Ag/Ag$^+$ nonaqueous reference electrode. Thin films of each polymer were prepared on gold-coated glass slides by dropcasting from the polymer tetrahydrofuran solution (1 mg/mL). The voltammograms

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are summarized in Figure 2. All polymers displayed broad oxidation peaks at 0.2−0.3 V and reduction peaks at −0.2−0.1 V (vs Ag/Ag^+). Oxidation of all polymers was onset at ∼0 V, which is lower than that of polyalkylthiophenes and higher than that of electropolymerized PEDOTs. This was due to the single oxygen electron donor on the thiophene ring, compared with none on polythiophenes and two on PEDOT.

One of the most challenging issues for conducting polymers as biomaterials is the electrostability in aqueous solution. It was reported that polypyrrole and polyaniline were less stable when applying electrical potential.[28,29] To access the electrochemical behaviors of OEG-containing polyoxythiophenes in aqueous environment and their long-term stability, weapplied poly(**1**)-Mg with a cyclic potential in aqueous solution containing 0.1 M $LiClO_4$ electrolyte at various scan rates (from 25 to 250 mV/s). Poly(**1**)-Mg was also applied with a cyclic potential in aqueous solution for 5000 cycles as shown in Figure 3B. Besides the initial scan, all later scans displayed almost identical cyclic voltammograms. This result supported the electrostability of our OEG-containing polyoxythiophenes in aqueous solution. The films also displayed high stability in buffer solution as shown in Figure 3C. The UV−visible spectra were almost identical after immersing the polymer film in PBS solution for 4 days

The conductivity of the polymers was assessed by an interdigitated microelectrode. The films were dropcasted onto the electrodes and then a 50 mV potential offset was applied between the electrodes during cyclic voltammetric scans. Poly(3-hexyl)thiophene was used as standard. The output current indicated that the polymer's conductivity were 1−0.01% of the poly(3-hexyl)thiophene (see Figure S3 in the Supporting Information). This was mainly due to the charge-localization from the cation complexation with the oligo-ethylene glycol side-chains.

In vitro Cell Compatibility. It is critical for the materials applicable for cell engineering platform or tissue culture scaffolds to be nontoxic. Although oligoethylene glycol groups are sometimes utilized to reduce protein or cell absorption on the surface, the number of repeating units of oligoethylene glycol is critical. We measured the contact angles of spincasted poly(**1**−**4**) films. When water droplet was dripped onto poly(**1**) film, the contact angle was measured as 61.7°. On the other hand, contact angle of poly(**2**) film was measured as 91.5° and contact angle of poly(**4**) film was measured as 43.6°. These films are more hydrophobic than those who hindered protein binding. Therefore, the cells could attach and proliferate. The cell cytotoxicity of poly(**1**−**4**) was assessed with NIH3T3 fibroblast cells by using spincoated poly(**1**−**4**) glass slides and uncoated glass slide as control experiment. The morphology of spincasted films are shown in Figure S4 in the Supporting Information. Comparing to typical electropolymerized films, the films spincasted were smooth with surface roughness at the range of 2 to 3 nm. The film thickness was ranging from 12 to 14 nm. Cell viability was determined by a standard MTT cell proliferation assay.[38] Figure 4A displayed the cell viability after 72 h incubation in DMEM buffer solution containing 10% bovine serum. All polymers showed no significant cytotoxicity to NIH3T3 cells. In addition, we also investigated NIH3T3 cell attachment and proliferation on the polymer film as a function of cell culture time (Figure 4B) comparing to standard tissue culture plate. Cells were attached to all polymers after 6 h incubation. As shown in the microscopy images (Figure 5), incubation attached cells are

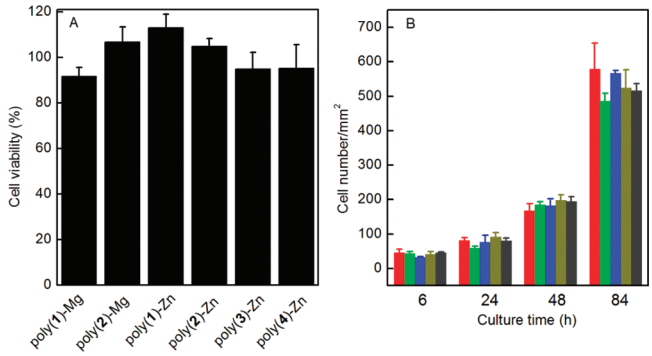

Figure 4. (A) Relative viability of NIH3T3 fibroblast cells measured by MTT assay on polyoxythiophene dropcasted films for 4 days. (B) Measured cell density of NIH3T3 fibroblast cells cultured on standard tissue culture plate (red), poly(**1**)-Mg (green), poly(**2**)-Mg (blue), poly(**3**)-Zn (brown), and poly(**4**)-Zn (black) films for various periods of time.

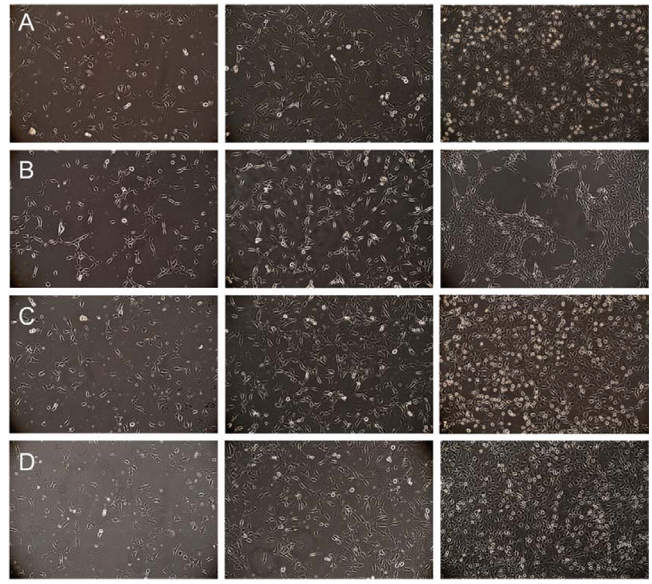

Figure 5. Microscopic images of NIH3T3 fibroblast cells cultured on (A) poly(**1**)-Mg, (B) poly(**2**)-Mg, (C) poly(**3**)-Zn, and (D) poly(**4**)-Zn coated glass slides after 24 h (left), 48 h (middle), and 84 h (right), respectively.

successfully grown to confluence on all polymer films after 84 h incubation. The cell culture medium was changed every other day during the whole experiment. The cells numbers grown on experimental and control groups on typical cell culture dishes were almost identical, further indicating that this newly developed OEG-containing polyoxythiophenes were cell compatible.

CONCLUSIONS

Methyl- and benzyl-capped oligoethylene glycol side-chain containing hydrophilic polyoxythiophenes are successfully synthesized by either Grignard metathesis (GRIM) polymerization or reductive coupling polymerization. The synthesized polyoxythiophenes are more hydrophilic comparing with traditional polyalkylthiophenes but not too hydrophilic to prevent cell attachment and proliferation. Polymers from different polymerization approaches display different colors after purification. Spectroelectrochemical studies confirm that

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the difference of color is from the difference of doping state. These oligoethylene glycol functionalized conducting polymers are highly electroactive and stable in aqueous environment even after applying cyclic potential for 5000 cycles. On the hand, the oligoethylene glycol groups are possibly bound to metal catalysts to prevent the formation of polymers with higher molecular weights. Their presence may also lead to charge localization. Therefore, less conductive polymers are obtained. In vitro toxicity studies show that all polymers exhibit no significant cytotoxicity toward NIH3T3 fibroblast cells. These cells on polyoxythiophene coated glass slides also show similar proliferation rate comparing to that on standard tissue culture plate. With the advantageous feature of higher hydrophilicity, lower operation potential, electrochemical stability in aqueous solution, and great cell viability, these oligoethylene glycol-containing polyoxythiophenes are promising for a variety of bioengineering and biomedical applications, including electrically conductive biointerface for stimulated cell engineering, coatings of bioelectrodes, and incorporation with other biodegradable polymer to fabricate electroactive tissue engineering scaffolds.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR and ^{13}C NMR spectra of the oligoethylene glycol-containing oxythiophene monomers, in vitro electrical conductivity measurements, and surface morphology. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Guimard, N. K.; Gomez, N.; Schmidt, C. E. *Prog. Polym. Sci.* **2007**, *32*, 876−921.
(2) Asplund, M.; Nyberga, T.; Inganäs, O. *Polym. Chem.* **2010**, *1*, 1374−1391.
(3) Wong, J. Y.; Langer, R.; Ingber, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3201−3204.
(4) Schmidt, C. E.; Shastri, V. R.; Vacanti, J. P.; Langer, R. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8948−8953.
(5) Garner, B.; Georgevich, A.; Hodgson, A. J.; Liu, L.; Wallace, G. G. *J. Biomed. Mater. Res.* **1999**, *44*, 121−129.
(6) Kotwal, A.; Schmidt, C. E. *Biomaterials* **2001**, *22*, 1055−64.
(7) Cen, L.; Neoh, G.; Li, Y.; Kang, E. T. *Biomacromolecules* **2004**, *5*, 2238−2246.
(8) Kim, S.; Oh, W. K.; Jeong, Y. S.; Hong, J. Y.; Cho, B. R.; Hahn, J. S.; Jang, J. *Biomaterials* **2011**, *32*, 2342−2350.
(9) Oren, R.; Sfez, R.; Korbakov, N.; Shabtai, K.; Cohen, A.; Erez, H.; Dormann, A.; Cohen, H.; Shappir, J.; Spira, M. E.; Yitzchaik, S. *J. Biomed. Mater. Sci., E* **2004**, *15*, 1355−1374.
(10) Whitehead, M. A.; Fan, D.; Akkaraju, G. R.; Canham, L. T.; Coffer, J. L. *J. Biomed. Mater. Res,. Part A* **2007**, *1*, 225−234.
(11) Wang, H.; Ji, Li; Li, D.; Wang, J. *J. Phys. Chem. B.* **2008**, *9*, 2671−2677.
(12) Whitehead, M. A.; Fan, D.; Akkaraju, G. R.; Canham, L. T.; Coffer, J. L. *J. Biomed. Mater. Res., Part A* **2007**, *1*, 225−234.
(13) Bayer, C. L.; Trenchard, I. J.; Peppas, N. A. *J. Biomed. Mater. Sci., Part E* **2010**, *21*, 623−634.
(14) Oh, W.; Kim, S.; Kwon, O.; Jang, J. *J. Nanosci. Nanotechnol.* **2011**, *11*, 4254−4260.
(15) Wangaman, M.; Sannigrahi, B.; Megeady, P.; Khan, I. M. *Eur. Polym. J.* **2003**, *39*, 1405−1412.
(16) Li, D.; Wang, H.; Fu, J.; Wang, W.; Jia, X.; Wang, J. *J. Phys. Chem.* **2008**, *112*, 16290−16299.
(17) Oh, W.; Kim, S.; Yoon, H.; Jang, J. *Small* **2010**, *6*, 872−879.
(18) Yang, J. H.; Kim, D. H.; Hendricks, J. L.; Leach, M.; Northey, R.; Martin, D. C. *Acta Biomater.* **2005**, *1*, 125−136.
(19) Green, R. A.; Lovell, N. H.; Poole-Warren, L. A. *Biomaterials* **2009**, *30*, 3637−3644.
(20) Svennersten, K.; Bolin, M. H.; Jager, E. W. H.; Berggren, M.; Richter-Dahlfors, A. *Biomaterials 30*, 6257−6264.
(21) Zelikin, A. N.; Lynn, D. M.; Farhadi, J.; Martin, I.; Shastri, V.; Langer, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 141−144.
(22) Mawad, D.; Gilmore, K.; Molino, P.; Wagner, K.; Wagner, P.; Office, D. L.; Wallace, G. G. *J. Mater. Chem.* **2011**, *21*, 5555−5560.
(23) Huang, L. H.; Hu, J.; Lang, L.; Wang, X.; Zhang, P. B.; Jing, X. B.; Wang, X. H.; Chen, X. S.; Lelkes, P. I.; MacDiarmid, A. G.; Wei, Y. *Biomaterials* **2007**, *28*, 1741−1751.
(24) Huang, L. H.; Zhuang, X. L.; Hu, J.; Lang, L.; Zhang, P. B.; Wang, Y. S.; Chen, X. S.; Wei, Y.; Jing, X. B. *Biomacromolecules* **2008**, *9*, 850−858.
(25) Rivers, T. J.; Hudson, T. W.; Schmidt, C. E. *Adv. Funct. Mater.* **2002**, *12*, 33−37.
(26) Guo, B.; Finne-Wistrand, A.; Albertsson, A. *Biomacromolecules* **2010**, *11*, 855−863.
(27) Brett Runge, M.; Dadsetan, M.; Baltrusaitis, J.; Ruesink, T.; Lu, L.; Windebank, A. J.; Yaszemski, M. J. *Biomacromolecules* **2010**, *11*, 2845−2853.
(28) Kros, A.; Sommerdijk, N. A. J. M.; Nolte, R. J. M. *Sens. Actuators, B* **2005**, *106*, 289−295.
(29) Yuan, Y. J.; Adeloju, S. B.; Wallace, G. G. *Eur. Polym. J.* **1999**, *35*, 1761−1772.
(30) Luo, S.-C.; Mohamed Ali, E.; Tansil, N. C.; Yu, H.-h.; Gao, S.; Kantchev, E. A. B.; Ying, J. Y. *Langmuir* **2008**, *24*, 8071−8077.
(31) Yokozawa, T.; Yokoyama, A. *Chem. Rev.* **2009**, *109*, 5595−5619. Osaka, I.; McCullough, R. D. *Acc. Chem. Res.* **2008**, *41*, 1202−1214.
(32) Yokozawa, T.; Yokoyama, A. *Chem. Rev.* **2009**, *109*, 5595−5619.
(33) Xue, C.; Luo, F.; Liu, H. *Macromolecules* **2007**, *40*, 6863−6870.
(34) Zhang, Q.; Jiao, L.; Shan, C.; Yang, G.; Xu, X.; Niu, L. *Syn. Met.* **2009**, *159*, 1422C1426.
(35) Cheng, Y.-J.; Luh, T.-Y. *J. Organomet. Chem.* **2004**, *689*, 4137−4148.
(36) Sheina, E. E.; Khersonsky, S. M.; Jones, E. G.; McCullough, R. D. *Chem. Mater.* **2005**, *17*, 3317−3319.
(37) Yamamoto, T.; Morita, A.; Miyazaki, Y.; Maruyama, T.; Wakayama, H.; Zhoa, Z.-h.; Nakamura, Y.; Kanbara, T.; Sasaki, S.; Kubota, K. *Macromolecules* **1992**, *25*, 1214−1223.
(38) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.