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Thiol Click Modification of Cyclic Disulfide Containing Biodegradable Polyurethane Urea Elastomers

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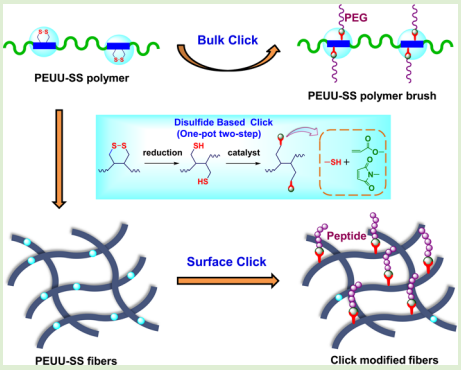
Supporting Information

ABSTRACT: Although the thiol click reaction is an attractive tool for postpolymerization modification of thiolmers, thiol groups are easily oxidized, limiting the potential for covalent immobilization of bioactive molecules. In this study, a series of biodegradable polyurethane elastomers incorporating stable cyclic disulfide groups was developed and characterized. These poly(ester urethane)urea (PEUU-SS) polymers were based on polycaprolactone diol (PCL), oxidized DL-dithiothreitol (O-DTT), lysine diisocyanate (LDI), or butyl diisocyanate (BDI), with chain extension by putrescine. The ratio of O-DTT:PCL was altered to investigate different levels of potential functionalization. PEG acrylate was employed to study the mechanism and availability of both bulk and surface click modification of PEUU-SS polymers. All synthesized PEUU-SS polymers were elastic with breaking strengths of 38−45 MPa, while the PEUU-SS(LDI) polymers were more amorphous, possessing lower moduli and relatively small permanent deformations versus PEUU-SS(BDI) polymers. Variable bulk click modification of PEUU-SS(LDI) polymers was achieved by controlling the amount of reduction reagent, and rapid reaction rates occurred using a one-pot, two-step process. Likewise, surface click reaction could be carried out quickly under mild, aqueous conditions. Furthermore, a maleimide-modified affinity peptide (TPS) was successfully clicked on the surface of an electrospun PEUU-SS(BDI) fibrous sheet, which improved endothelial progenitor cell adhesion versus corresponding unmodified films. The cyclic disulfide containing biodegradable polyurethanes described provide an option for cardiovascular and other soft tissue regenerative medicine applications where a temporary, elastic scaffold with designed biofunctionality from a relatively simple click chemistry approach is desired.



1. INTRODUCTION

Synthetic biodegradable elastomers (with chemical or physical cross-linking) can possess attractive properties for a broad variety of biomedical applications.[1,2] Biodegradable polyurethanes (PUs) are being explored for numerous applications in medicine, including but not limited to regenerative medicine, drug delivery systems, and implantable bioelectronics,[3−6] where biomaterials scientists are leveraging the diversity possible in PU chemistry to achieve desired mechanical, degradation, and biological behavior. A diverse range of physical properties from aliphatic diisocynate-based polyester urethanes can be achieved by employing a variety of soft segment constituents (polyester or polyester-ether macrodiol) and chain extenders (diol-, diamine-, or enzyme-sensitive peptide) that participate in hard segments.[1,3] After further processing (e.g., thermally induced phase separation, salt-leaching, electrospinning), biodegradable PU elastic porous scaffolds can be designed to approximate the compliance and other physical properties important for implantation in soft tissues and to facilitate the transfer of mechanical stimuli to indwelling cells.[1,7] Several recent reports have advanced the development of PUs with pendant reactive groups, including amino, carboxyl, hydroxyl, alkene, alkyne, and azide,[7−12] to covalently graft bioactive

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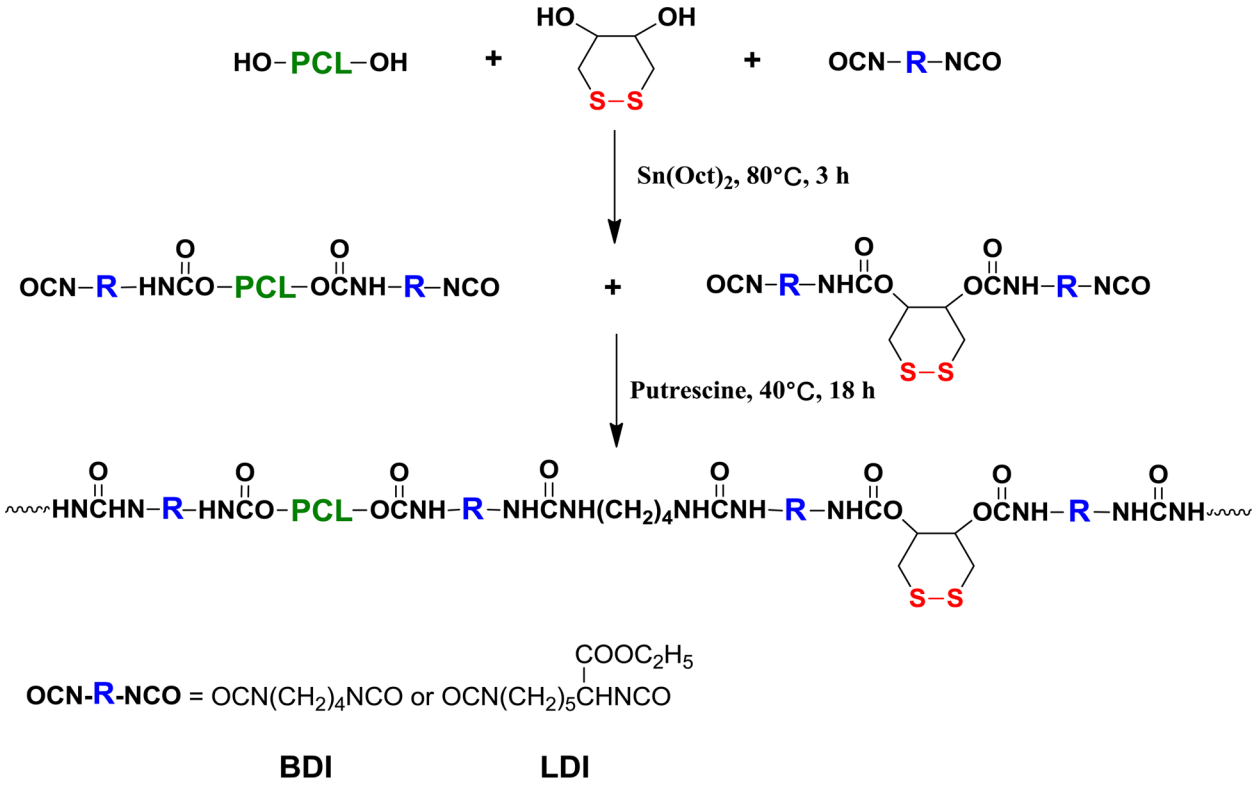
Scheme 1. Synthesis Route for PEUU-SS Polymers



molecules or drugs for biomedical and pharmaceutical applications.[13−15]

Click chemistry constitutes a class of reactions broadly characterized by efficiency, versatility, selectivity, and amenability to ambient, aqueous conditions.[16] Selection of "clicked on" functional groups allows the application of appropriate base polymers to be imparted with a broad variety of activities that may be desirable in materials science and particularly in biomedicine.[17−19] Of note, alkyne- and azide-containing PUs have been developed for further click modification by copper(I)-catalyzed azide−alkyne cycloaddition (CuAAC) reaction,[10,11] although residual copper catalyst toxicity may represent a concern for applications in some biological systems. An alternative click process, the thiol−ene reaction, in both its radical and base/nucleophilic forms, has also been exploited for site-specific functionalization. Several thiol-containing polymers including PUs, poly(methyl methacrylates), polyesters, chitosan, cellulose acetate, and polyamides have been reported recently.[20−25] A series of molecules including biotin, DNA, proteins, and pharmaceutical agents have been attached to thiolated polymers or surfaces by thiol-X chemistry.[26−30] PUs containing free thiol groups in the hard segment have been reported with protected thiols requiring subsequent deprotection and with thiolation of polyepichlorohydrin (PECH) used as soft segments, respectively.[20] Thiols in these polymers have further been nitrosothiolated for NO release; however, thiol-containing polymers are generally difficult to synthesize and require careful attention to reaction and storage conditions due to their propensity for oxidization.[20,22] Furthermore, the rapid formation of disulfides in thiol-containing polymers can render them insoluble in organic solvents and prevent characterization (e.g., molecular weight determination by GPC) and processing in the presence of oxygen, limiting their applicability and the implementation of thiol-click chemistry.

With the objective of addressing this limitation, elastic biodegradable poly(ester urethane)ureas (PEUUs) were synthesized from poly(caprolactone) diol (for hydrolytic lability), butyl or lysine diisocyanate, and putrescine as a chain extender. By variably adding oxidized DL-dithiothreitol in the synthesis as a competing diol, a series of polymers with stable disulfide groups (PEUU-SS) were generated. To evaluate the availability of cyclic disulfide groups in PEUU-SS for click functionalization, PEG acrylate was used as a model molecule for surface and bulk modification. To evaluate the potential for incorporating a bioactive surface molecule, the peptide TPSLEQRTVYAK, which has been shown to support endothelial progenitor cell (EPC) attachment,[31,32] was end-modified with maleimide and applied to an electrospun PEUU-SS surface, and this modified PEUU-SS surface was contacted with EPC to enhance acute cell adhesion.

2. MATERIALS AND METHODS

2.1. Materials. Polycaprolactone diol (PCL, M_n = 2000), 1,4-diisocyanatobutane (BDI), putrescine, stannous octotate ($Sn(Oct)_2$), *trans*-4,5-dihydroxy-1,2-dithiane (O-DTT), DL-dithiothreitol (DTT), tributylphosphine (Bu_3P), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), poly(ethylene glycol) methyl ether acrylate (PEG acrylate, M_n = 480), fluorescein isothiocyanate isomer I (FITC), 5(6)-carboxyfluorescein *N*-hydroxysuccinimide ester (fluorescein-NHS), anhydrous dimethyl sulfoxide (DMSO), and all other chemicals and solvents, unless otherwise specified, were purchased from Sigma-Aldrich. L-Lysine ethyl ester diisocyanate (LDI) was purchased from Infine Chemicals (Shanghai, China). Alexa Fluor 568 phalloidin, 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), and Alexa Fluor 488 C_5 maleimide were purchased from Thermo Fisher Scientific.

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PCL was dried in a vacuum oven at 60 °C overnight to remove residual water before synthesis. LDI, BDI, and putrescine were purified using vacuum distillation before usage.

2.2. Synthesis of PEUU-SS Polymers. Poly(ester urethane)ureas with disulfide groups (PEUU-SS) were synthesized by a two-step solution polymerization (Scheme 1). The stoichiometry of (O-DTT with PCL):(LDI or BDI):putrescine was 1:2:1. In a typical example, under argon protection, PCL diol (10 g, 5 mmol) and O-DTT (0.76 g, 5 mmol) were dissolved in DMSO (100 mL) in a three-necked flask, and LDI (4.01 mL, 20 mmol) was added dropwise under magnetic stirring, followed by the addition three drops of $Sn(Oct)_2$. The prepolymerization reaction was allowed to proceed for 3 h at 80 °C. Afterward, the reaction solution was cooled to room temperature, and putrescine (1.01 mL, 10 mmol) in DMSO (130 mL) solution was added dropwise; then, the reaction continued for another 18 h at 40 °C. The resulting polymer was precipitated in deionized water (DI water), then immersed in isopropanol for further purification and dried under vacuum. Yields were in the range of 85−92%.

Different numbers of disulfide groups were incorporated into the PEUU polymers by controlling the molar ratios of O-DTT:PCL (1:1, 2:1, 3:1) in the prepolymerization step. The polymers are abbreviated as PEUU-SS(X)*n*, where X refers to the type of diisocyanate used (LDI = L-lysine ethyl ester diisocyanate, BDI = 1,4-diisocyanatobutane) and *n* refers to the molar ratio of O-DTT:PCL applied in the step of prepolymerization (Table 1).

Table 1. Molar Ratios of Reaction Reagents, Theoretical Molecular Weight of Repeating Unit, Soft Segment Content, and Theoretical Disulfide Density in the Designed PEUU-SS Polymers

polymers	molar ratio of PCL:O-DTT:LDI or BDI:putrescine	theoretical molecular weight of repeating unit (g/mol)	soft segment content (wt %)	disulfide density (mmol/g)
PEUU-SS(LDI)1	1:1:4:2	3233	62	0.31
PEUU-SS(LDI)2	1:2:6:3	3926	51	0.51
PEUU-SS(LDI)3	1:3:8:4	4619	43	0.65
PEUU-SS(BDI)1	1:1:4:2	2889	69	0.35

2.3. Synthesis of Functional Peptide TPS-Maleimide. Maleimide-TPSLEQRTVYAK (TPS-maleimide) was synthesized with a 6-maleimidohexanoic acid linked on the N-terminus of TPS peptide (Scheme S1 in the Supporting Information) and commercially synthesized by GL Biochem (Shanghai) (China) through solid-phase peptide synthesis technology. The resultant peptides were characterized by high-performance liquid chromatography and mass spectrometry analysis with a 95% purity.

2.4. Characterization. Polymer chemical structure was characterized by ^{1}H nuclear magnetic resonance (^{1}H NMR, 300 MHz, Bruker Biospin, Billerica, MA) using DMSO-d_6 as a solvent. Fourier transform infrared (FTIR) spectra were recorded on a Thermo Nicolet iS10 spectrometer equipped with a diamond Smart iTR. Polymer surface composition was analyzed by X-ray photoelectron spectroscopy (XPS, Physical Electronics PHI 5802 equipped with a monochromatic Al Kα source). Thermal properties were measured by differential scanning calorimetry (DSC, DSC-60, Shimazu) at a scanning range of −100 to 200 °C at a rate of 10 °C/min under nitrogen flow, and the second cycle was recorded. Wide-angle X-ray diffraction (WAXD) experiments were carried out on PU films using a D/max-2550 PC XRD instrument (Rigaku, Japan) with a Cu Kα source. The surface hydrophilicity of films was studied by measuring water contact angle (WCA) using a sessile drop method with distilled water (OCA40, Dataphysics, Germany). Water absorption was defined in terms of the difference of the wet mass (w_2) and dry mass (w_1) of the film (10 × 10 × 0.15 mm, n = 3) and calculated as water absorption ratio (%) = $100 \times (w_2 - w_1)/w_1$. As an initial assessment of PEUU-SS polymer cytocompatibility, the proliferation of primary rat smooth muscle cell (rSMC) growth on the surface of representative PEUU-SS(LDI)1 cast films was performed with live/dead cell staining at days 2, 4, and 6 and with employment of a mitochondrial activity assay.[7]

2.5. Polymer Processing by Film Casting and Electrospinning. Cast films (∼150 μm thick) were prepared by casting 5.0% w/v polymer solutions in 1,1,1,6,6,6-hexafluoroisopropanol (HFIP) onto a polytetrafluoroethylene plate, followed by solvent evaporation. Nanofibrous sheets were fabricated by electrospinning (ES). PEUU-SS(BDI)1 was dissolved in HFIP (15% w/v) at RT overnight. The solution was fed at 1.5 mL/h by syringe pump (Harvard Apparatus, United States) through a stainless-steel capillary (1.2 mm inner diameter) located 15 cm over an aluminum collector plate. Two high-voltage generators (Gamma High Voltage Research, United States) were employed to charge the steel capillary to 12 kV and the collector to −4 kV, respectively.

2.6. Mechanical Properties. For uniaxial tensile testing, dumbbell-shaped strips (2.5 × 20 mm, n = 4) were cut from the polymer cast films and mechanical properties were measured on an MTS Tytron 250 MicroForce testing workstation at room temperature with a crosshead speed of 25 mm/min according to ASTM D638-98. Permanent deformation and cyclic tensile testing (10 cycles) at low deformation (30%) and high deformation (400%) were performed as previously described.[33]

2.7. Polymer Degradation. Polymer degradation behavior after exposure to aqueous and enzymatic environments was quantified by dry weight loss. The polymer cast films (10 × 10 × 0.15 mm) were immersed in 10 mL of PBS (pH 7.4) containing 0.2 mg/mL sodium azide or with 100 U/mL lipase (Sigma, from *Thermomyces lanuginosus*) or 0.3 mg/mL elastase (Sigma, Type I, from porcine pancreas), and the degradation was conducted at 37 °C in a water bath with the solution exchanged every 7 days. At each time point, the samples (n = 3) were rinsed with DI water (three times) and dried in a vacuum oven, followed by weighing. The mass remaining (%) was calculated to be $W_1/W_0 \times 100\%$, where W_0 and W_1 are the weights of films before and after degradation, respectively.

2.8. Bulk Thiol Click Modification by PEG Acrylate. The bulk (B) click modification of PEG acrylate was a one-pot, two-step process (Scheme 2). For example, PEUU-SS(LDI)1 polymer (3.233 g, containing 1 mmol disulfide groups) was dissolved in DMF (5% w/v), then Bu_3P (1.7 equiv to disulfide) was added to reduce the disulfide for 2 h at room temperature. Afterward, PEG acrylate (7 equiv disulfide) was added directly for another 2 h modification. The resulting PEUU-SS(LDI)1-PEG(B) was precipitated in ether to remove Bu_3P, further immersed in DI H_2O/ethanol (1:1 v/v) to remove PEG acrylate, and freeze-dried. Yields were 80%. In a similar way, PEUU-SS(LDI)2-PEG(B) and PEUU-SS(LDI)3-PEG(B) were obtained by bulk modification of PEUU-SS(LDI)2 and PEUU-SS(LDI)3 with PEG acrylate, respectively. The theoretical density of disulfide in the developed PUs was calculated as $(W/uM_w^{theo})*n$, where W is the weight of PU, uM_w^{theo} is the theoretical molecular weight of one polymer unit, and n is the number of disulfides in the polymer unit (also the molar ratio of O-DTT to PCL) (Table 1).

To further quantify the controllability of bulk click modification of PEUU-SS polymers in the presence and absence of air, we chose PEUU-SS(LDI)1 for PEG acrylate modification by the previously mentioned protocols, while the reagent molar ratios were tuned as disulfide/Bu_3P/PEG acrylate = 1:n:2n (n = 0.25, 0.5, 1, 1.1, 1.7); all reactions were performed in the presence of air. Except for the molar ratio of disulfide/Bu_3P/PEG acrylate = 1:1:2 these reactions were also performed in the absence of air.

2.9. Surface Disulfide Density Determination and Surface Thiol Click Modification. The surface disulfide density was determined by measuring the thiol groups formed after disulfide reduction with TCEP. Thiol group determination was performed using Ellman's reagent. The procedure was adapted from previously published methods with slight modification.[34,35] Briefly, PEUU-SS cast films were reduced by 20 mM TCEP solution in DI water for 1 h at room temperature with shaking, then washed by DI H_2O three times and PBS three times, followed by prompt incubation of 1 mM

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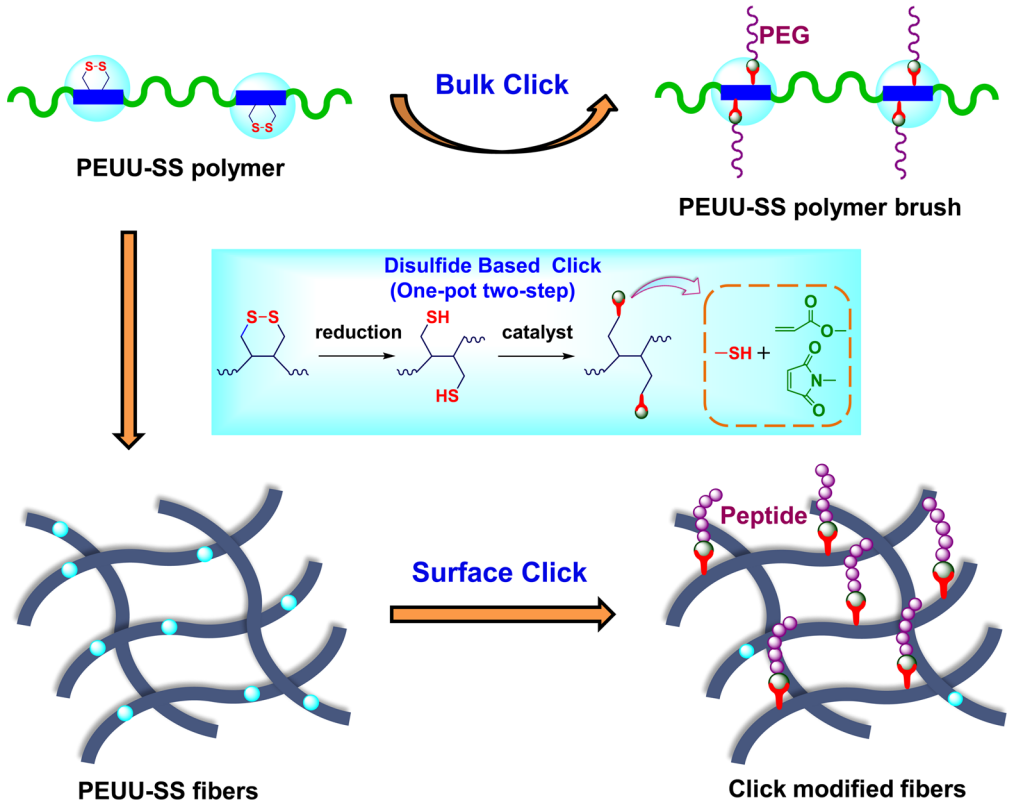
Scheme 2. Bulk Click Modification of PEUU-SS Polymers and Surface Click Modification on PEUU-SS Films



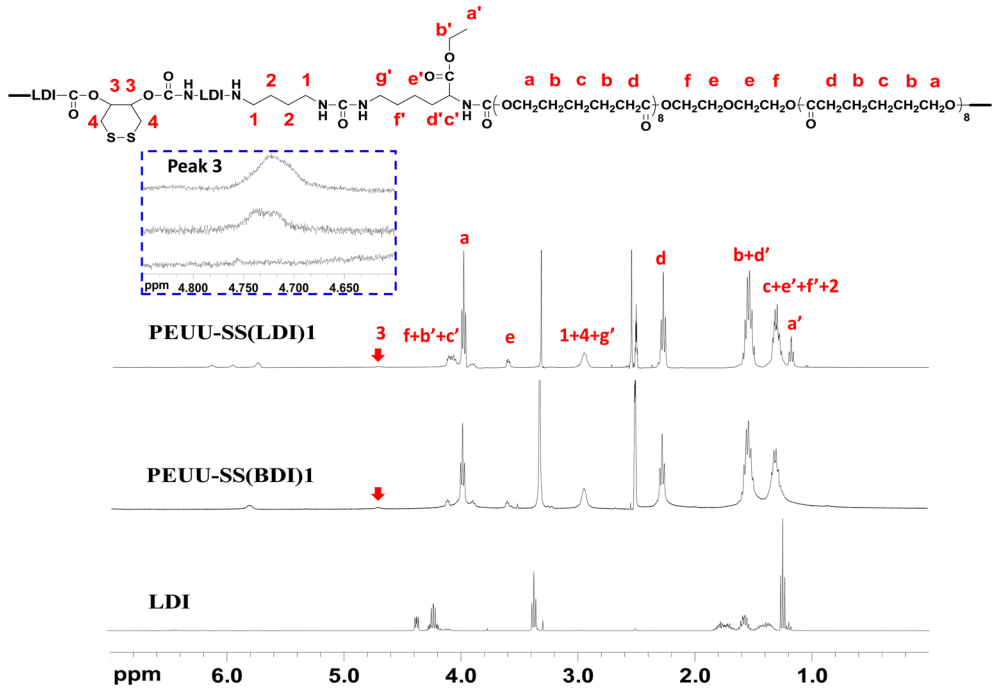


Figure 1. ^{1}H NMR spectra of PEUU-SS(LDI)1, PEUU-SS(BDI)1, and LDI; DMSO-d_6 as solvent.

Ellman's reagent in phosphate buffer (0.1 M sodium phosphate, 1 mM EDTA, pH 8.0) for 15 min at room temperature. Absorbance at 412 nm was measured and concentration values were obtained from comparison of measurements to a standard curve generated from DL-dithiothreitol dilutions in phosphate buffer.

PEUU-SS(LDI) cast films were also click reacted with PEG acrylate. The previously mentioned protocols were used for the reduction of disulfide groups on the surface of films, followed by incubation with PEG acrylate (100 mg/mL in DI water) for 2 h at room temperature. The films were then washed by DI water several times and freeze-dried.

2.10. Surface Functionalization of ES Fibers with TPS Peptide and Fluorophore. In pilot experiments, TPS-maleimide (1 mg/mL) and Alexa Fluor 488 C_5 maleimide (0.01 mg/mL) were clicked on the surface of ES PEUU-SS(BDI)1 fibers (Scheme 2). The polymer surface composition was analyzed by XPS. Further

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confirmation of TPS peptide attachment was made by conjugating the free amino group of lysine in the peptide with 0.5 mg/mL fluorescein-NHS solution (0.2 M $NaHCO_3$, pH 8.3) for 24 h at 4 °C in the dark. The obtained fluorescein-labeled ES membrane was immersed in DI water and rinsed three to four times daily with DI water and PBS over 3 days at room temperature to remove physically absorbed fluorescein. Unmodified ES films were generated similarly as a control, with the exclusion of the reducing (TCEP) step. Fluorescence images were taken by fluorescence microscopy (Eclipse Ti, Nikon).

2.11. EPC Cell Isolation and Short-Term Adhesion Assay. Mouse bone-marrow-derived EPCs were isolated and cultured in endothelial basal medium-2 (Lonza) supplemented with EGM-2MV single aliquots (Lonza) according to previous protocols.[36] After 7 days of culture, EPCs were characterized and used for further analysis. The cultured EPCs were identified by flow cytometry analysis and immunostaining with stem cell markers CD34 and CD133, endothelial lineage markers VEGF receptor-2 (VEGFR-2, flk-1) and VE-cadherin, and hematopoietic cell marker CD45.

ES PEUU-SS(BDI)1 sheets (1.4 cm diameter) with and without TPS modification were placed in 24-well tissue culture polystyrene plates and sterilized with 70% ethanol immersion and washed with PBS several times. EPCs suspended in serum-free M199 medium were seeded at a concentration of 5×10^4 cells/mL. After incubation for 1 h at 37 °C with 5% CO_2 in a humidified atmosphere, the medium was aspirated, and cells loosely attached were removed by three rinses. The mitochondrial activity of adhered EPCs was measured by CCK-8 assay (Invitrogen) according to the manufacturer's protocol. For each group, four samples were used in parallel. On a separate set of sheets, the attached cells were fixed with 4% paraformaldehyde and stained with Alexa Fluor 568 phalloidin and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI). Specimens were observed under confocal laser scanning microscopy (Carl Zeiss LSM 700, Jena, Germany).

2.12. Statistical Analyses. All results are represented as mean ± standard deviation. The data were analyzed by one-way ANOVA, followed by Tukey's test for the evaluation of specific differences with Origin Pro 8. $*p < 0.05$, $**p < 0.01$ were considered to represent significant differences.

3. RESULTS

3.1. Polymer Characterization. The chemical structures of polymers were confirmed by ^{1}H NMR analysis. The ^{1}H NMR spectra of PEUU-SS(LDI)1, PEUU-SS(BDI)1 is seen in Figure 1. PEUU-SS(LDI)1 showed a strong signal at 1.38 ppm assigned to methyl protons in the LDI groups. The peaks of O-DTT (peak 3) also weakly showed in the spectra of the two polymers.

DSC data provided the glass-transition and crystalline properties of PEUU-SS polymers (Figure 2). All polymers showed a T_g at approximately −60 °C. PU-SS(BDI)1 showed the peak of T_m at 20 °C, while PEUU-SS(LDI) polymers did not show obvious melting peaks of the soft segment, illustrating that the PEUU-SS(LDI) polymers were more amorphous than PEUU-SS(BDI) polymers. The crystallinity of polymers was further confirmed by WAXD (Figure 3). Peaks at 2θ = 21.6 and 24° corresponded to the diffraction of the 110 and 200 lattice plane of the orthorhombic crystalline PCL.[37] PEUU-SS(LDI) polymers only showed the broad peak at 21.6°, which decreased at low soft segment concentration, whereas the shoulder peak at 24° was much stronger in the PEUU-SS(BDI)1.

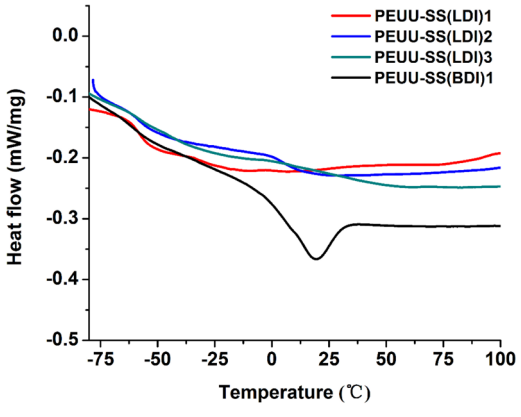

Figure 2. DSC analysis (second cycle) of cast PEUU-SS polymer films.

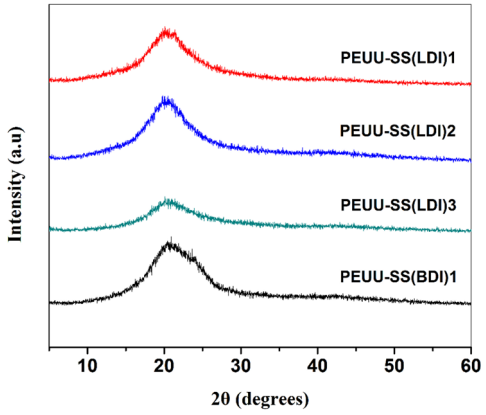

Figure 3. Wide-angle X-ray diffraction (WAXD) profiles of PEUU-SS polymers.

Representative tensile stress−strain curves of the PEUU-SS cast films are shown in Figure 4A, with average mechanical parameters summarized in Table 2. The strain at break of PEUU-SS(LDI) polymers decreased with increasing hard segment content, from 797% for PEUU-SS(LDI)1 to 680% for PEUU-SS(LDI)3 ($p < 0.05$), while the data were similar in tensile strength (38 to 45 MPa), initial modulus (2.9 to 3.4 MPa), and 100% modulus (1.4 to 1.9 MPa). PEUU-SS(BDI)1 showed tensile strain and strength at 830% and 40 MPa, respectively, close to the values for PEUU-SS(LDI)1, while initial modulus and 100% modulus were significantly higher than for PEUU-SS(LDI)1 and the permanent set was much higher (Figure 4B−D).

To further understand the elasticity of the materials, we performed cyclic tensile loading with a maximum strain of 30 (Figure 5A) or 400% (Figure 5B). Most of the PEUUs exhibited a large hysteresis loop in the first cycle, followed by much smaller hysteresis loops in the next nine cycles. At 30% low deformation, most of the samples showed a small unrecoverable deformation (∼5%). At the large strain of 400%, the unrecoverable deformations became much more appreciable (∼100%) for PEUU-SS(LDI) polymers, and PEUU-SS(BDI)1 was >200%.

Polymer degradation was evaluated in PBS, lipase, and elastase solutions at 37 °C (Figure 6). PEUU-SS polymers showed minimal degradation both in PBS over the 16 week period and elastase solution over the 8 week period, whereas significantly higher mass loss occurred in lipase solution ($p < 0.05$). In particular, the mass remaining for PEUU-SS(LDI)1 samples was 86% after 8 weeks of degradation in lipase solution, while the PEUU-SS(BDI)1 samples had markedly more degradation to 48% at the same time point.

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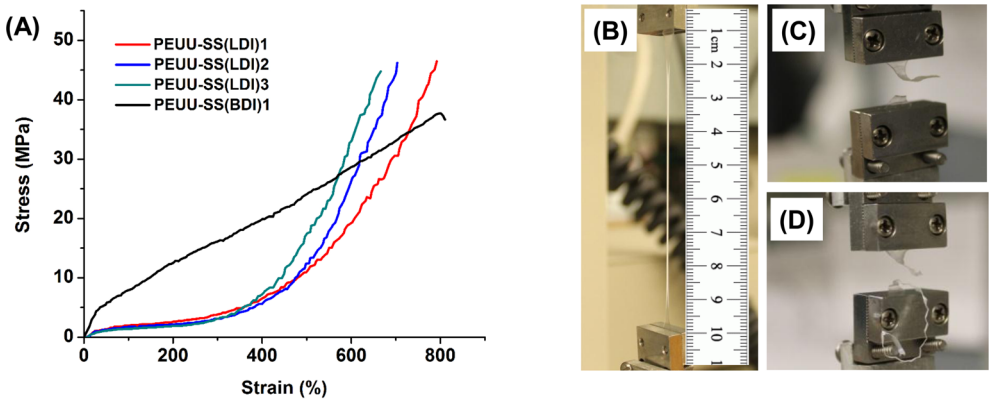

Figure 4. (A) Typical stress−strain curves and (B−D) permanent deformations of cast films of PEUU-SS polymers. (B) Typical image showing the large elongation of the polyurethanes before break. (C) PEUU-SS(LDI)1 showed relatively small permanent set. (D) PEUU-SS(BDI)1 showed relatively large permanent set.

Table 2. Summarized Mechanical Properties of PEUU-SS Polymers[a]

polymer	strain at break (%)	tensile strength at break (MPa)	initial modulus (MPa)	100% modulus (MPa)	permanent set (%)
PEUU-SS(LDI)1	797 ± 20[a]	38.7 ± 3.2[a]	3.3 ± 0.3[a]	1.9 ± 0.2[a]	50 ± 9[a]
PEUU-SS(LDI)2	720 ± 11[b]	45.0 ± 2.6[a]	2.9 ± 0.3[a]	1.6 ± 0.1[a]	52 ± 14[a]
PEUU-SS(LDI)3	680 ± 20[b]	38.1 ± 4.5[a]	3.4 ± 0.8[a]	1.4 ± 0.1[a]	53 ± 12[a]
PEUU-SS(BDI)1	830 ± 41[a]	40.0 ± 2.7[a]	18.4 ± 1.5[b]	8.5 ± 0.6[b]	650 ± 30[b]

[a]a and b denote statistically distinct groups for each measured parameter, $p < 0.05$.

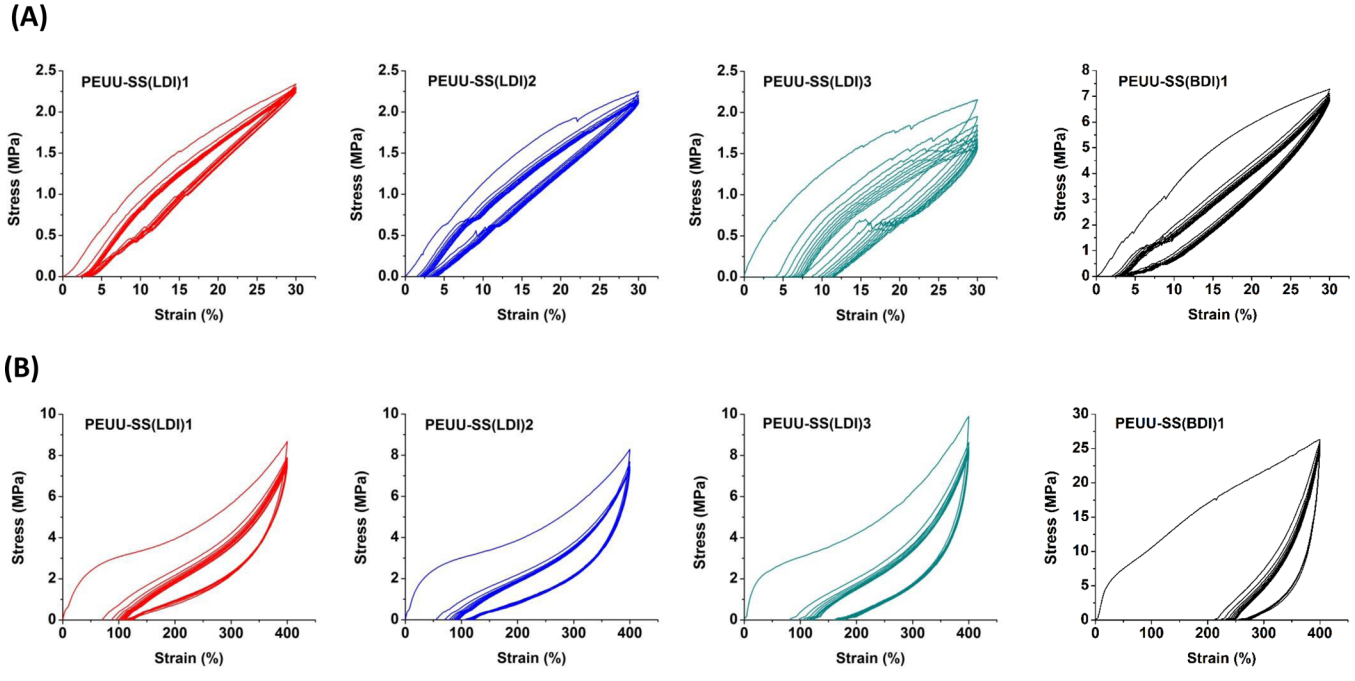

Figure 5. Cyclic tensile response curves for 10 cycles at (A) low deformation (30%) and (B) high deformation (400%) for PEUU-SS polymers.

As an initial assessment of PEUU-SS polymer cytocompatibility, the proliferation of primary rat smooth muscle cell (rSMC) growth on the surface of representative PEUU-SS(LDI)1 cast films showed that rSMCs proliferated quickly and reached confluence over the 6 day study (Figure S1A in the Supporting Information), with no visualization of dead cells. In support of this visual trend, the MTS assay (Figure S1B in the Supporting Information) found increasing metabolic activity. The polymer film was less supportive of rSMC adhesion and proliferation than tissue culture polystyrene.

3.2. Bulk and Surface Click Modification of PEUU-SS Cast Films. PEG acrylate was used as a model molecule to study the availability and mechanism of bulk and surface click modification on PEUU-SS polymers. LDI- and BDI-based PEUU-SS polymers showed different physical and chemical characteristics based on the characterization previously mentioned. PEUU-SS(LDI) polymers showed better organic solubility. Thus, bulk click mechanism was studied with PEUU-SS(LDI) polymers.

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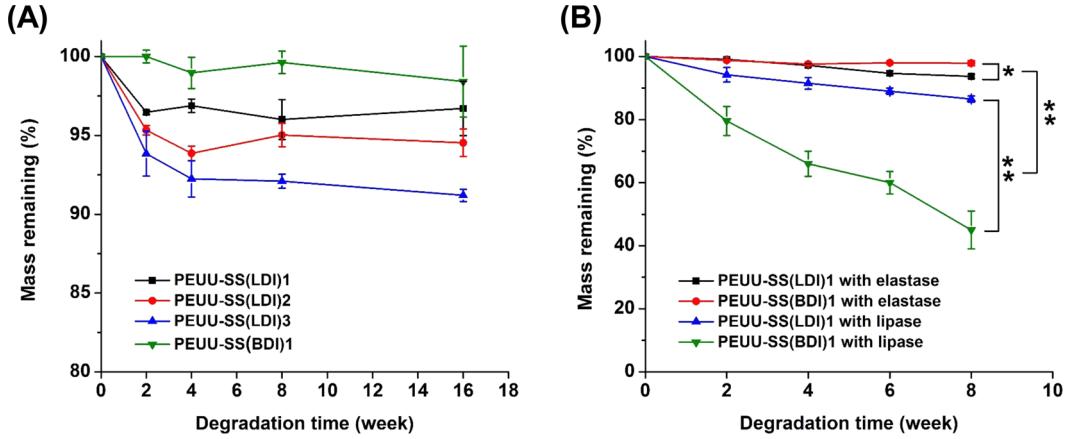

Figure 6. Mass remaining for PEUU-SS cast films in (A) PBS and (B) enzymatic solutions at 37 °C. *$p < 0.05$, **$p < 0.01$.

The chemical structure of PEUU-SS(LDI)1 by PEG bulk modification and quantitatively controlled modification was confirmed by the ^{1}H NMR spectra (Figure 7). The chemical peaks 5′(−OC$\underline{H}_2$-C$\underline{H}_2$-, 4H, 3.51 ppm) and 7′(−OC$\underline{H}_3$, 3.24 ppm) belonging to PEG acrylate were shown in the spectra of PEUU-SS(LDI)1-PEG(B), while there were no signals attributable to unreacted double bonds (peaks 1′ and 2′, 6.0−6.5 ppm),[38] confirming the click reaction of PEG acrylate onto the disulfide groups. By calculating the ratio of peak d in PCL to peak 7′ in PEG acrylate, the yield of this click modification was ∼100% (the content of S−S or −SH value was estimated by theoretical values, Table 1). ^{1}H NMR of PEUU-SS(LDI)2 and PEUU-SS(LDI)3 with PEG acrylate bulk modification also confirmed that these polymers could be modified at high conversion (Figure S2 in the Supporting Information). The bulk click modification in the presence and absence of air and with varying presence of the reduction agent Bu_3P is shown in Figure 7B. It was found that the amount of PEG clicked onto the polymer could be controlled by varying the amount of the reducing agent, Bu_3P, present. This is seen in comparing the ratio of integrated areas for peak 7′ in PEG acrylate to peak d in PCL, which increased from 0.05, 0.09, to 0.17 in concert with increases in the Bu_3P content. As the disulfide groups become fully reduced, no further PEG attachment is seen. Performing the modification in the absence of air showed integration levels that were not markedly different than when air was present.

For polymer surface modification, the S−S density on the surface of cast films of PEUU-SS polymers was quantified by Ellman's test (Table 3), with DL-dithiothreitol used as standard. The disulfide density increased from 3.4×10^{-2} nmol/mm^2 for PEUU-SS(LDI)1 to 4.5×10^{-2} nmol/mm^2 for PEUU-SS(LDI)3. ATR-FTIR of bulk and surface click-modified PEUU-SS polymers confirmed the click modification by PEG acrylate (Figure 8). The specific peak belonging to PEG at 1242 cm^{-1} (C−O−C) became stronger after surface and bulk click modification, while much stronger signals were found for the latter. Furthermore, the signal appeared to increase from PEUU-SS(LDI)1 to PEUU-SS(LDI)3 with corresponding modifications. Water contact angle (Figure 9A) and water absorption (Figure 9B) further confirmed the PEG click modification by the impact on polymer hydrophilicity. Water contact angle was consistently decreased with PEG surface and bulk modification, without marked differences between surface and bulk modification. There was also not an obvious dependence on the amount of disulfide in the hard segment. For water absorption, there was a clear difference between surface and bulk modification with PEG (Figure 9B). Water absorption of cast films of PEUU-SS polymers with PEG surface modification did not show significant changes versus the corresponding unmodified films. For bulk PEG modification there was a clear effect on water absorption that increased with increasing disulfide content in the base polymer ($p < 0.05$).

3.3. Surface Functionalization of ES Fibers. PEUU-SS(BDI)1 was electrospun into a fibrous sheet to evaluate the ability of the PEUU-SS polymers to be processed and functionalized with a bioactive moiety. Electron microscopy showed distinct fibers resulting from the electrospinning process (Figure 10A). Fluorescence images of ES PEUU-SS(BDI)1 fibers demonstrated successful modification with Alexa Fluor 488 C_5 maleimide (Figure 10B,C). After TPS-maleimide click modification, the PEUU-SS(BDI)1-TPS(S) fibers maintained their morphology (Figure 10D). The amino groups on the peptide-modified surface of the fibers after TPS modification could be used for fluorescein-NHS labeling, as indicated by the green fluorescence shown in Figure 10E, while no signal was detected for the control (Figure 10F). The fluorescence intensity was still apparent after 3 days of immersion in and periodic washing by DI water and PBS (Figure S3 in the Supporting Information). XPS spectra (Figure S4 in the Supporting Information) of ES PEUU-SS(BDI)1 and ES PEUU-SS(BDI)1-TPS(S) and the resulting surface composition (Table S1 in the Supporting Information) showed an increase in surface nitrogen from 3.0 to 4.6%, further confirming the modification.

3.4. Effect of TPS Modification on EPC Adhesion to ES Fibrous Sheets. The enhancement of attachment of EPCs on functionalized surfaces was evaluated by comparing EPC attachment on ES PEUU-SS(BDI)1 and ES PEUU-SS(BDI)-TPS(S) fibrous sheets (Figure 11). As represented by metabolic activity and qualitatively confirmed with visualization, EPC acute adhesion was greater on ES PEUU-SS(BDI)-TPS(S) compared with nonmodified sheets (Figure 11A−C).

4. DISCUSSION

A wide variety of biofunctional covalent modifications have been pursued on synthetic polymer surfaces since the early reports of Massia and Hubbell in 1990 showing the ability of GRGD attachment to improve the cell adhesive properties of

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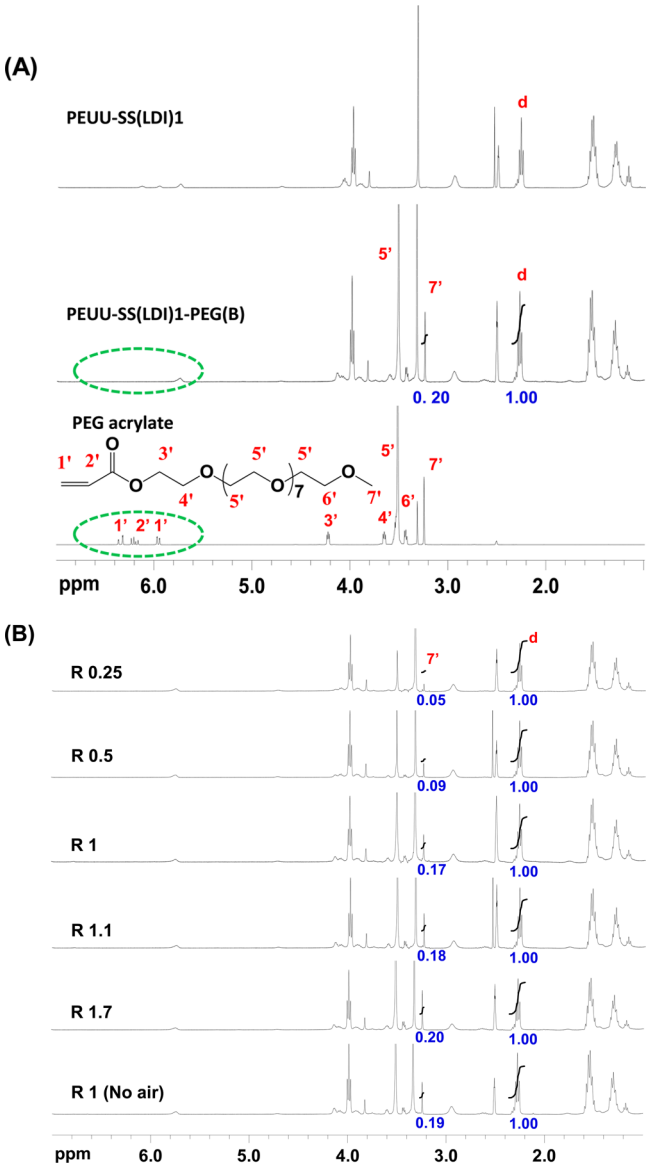

Figure 7. (A) ^{1}H NMR spectra of PEUU-SS(LDI)1, PEUU-SS(LDI)1-PEG(B), and PEG acrylate (M_n = 480). (B) ^{1}H NMR spectra of quantitatively modified PEUU-SS(LDI)1 with PEG acrylate by tuning the molar ratio (R) of the reduction reagent (Bu_3P) to disulfide groups in the presence of air. R1 (no air) was performed in the absence of air. DMSO-d_6 as solvent.

Table 3. Surface Disulfide Density on PEUU-SS Cast Films by Ellman's Test, DL-Dithiothreitol Was Used As Standard

polymers	surface disulfide density (*10^{-2} nmol/mm^2)
PEUU-SS(LDI)1	3.4 ± 0.5
PEUU-SS(LDI)2	4.1 ± 0.3
PEUU-SS(LDI)3	4.5 ± 0.5
PEUU-SS(BDI)1	3.8 ± 0.1

poly(hydroxyl ethyl methacrylate) and poly(ethylene terephthalate).[39]

As the general approach of covalently linking biomolecules to the surface and bulk of polymers has expanded, the array of chemistries employed and polymer substrates modified have expanded. In this report, we sought to extend this concept to thermoplastic biodegradable elastomers that are applicable to a

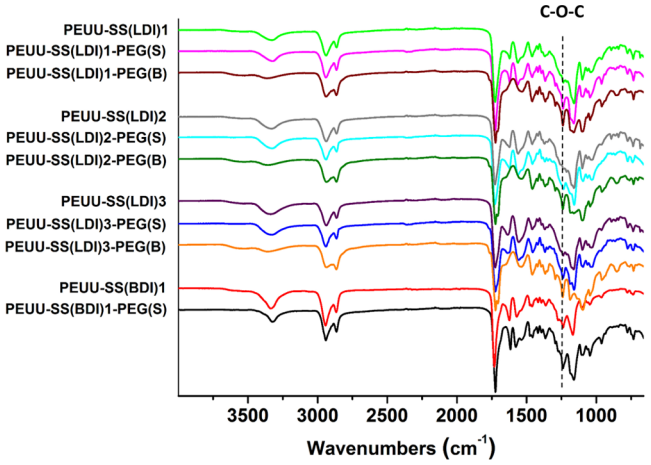

Figure 8. ATR-FTIR of PEUU-SS cast films and corresponding cast films with PEG surface (S) and bulk (B) click modification.

variety of scaffold applications in soft tissue repair, including cartilage, abdominal wall, blood vessel, heart valve, and cardiac patch.[1,40] It was also desired to employ the specificity and ease associated with thiol-based click chemistry.

Thiol-based click reactions generally provide a powerful approach to synthesize branched molecules and networks or to engineer multifunctional surfaces with high efficiency and rapid kinetics, insensitivity to oxygen or water, a lack of potentially toxic catalysts, and high selectivity.[26] While some limitations still exist in thiol-click chemistries,[20,22,41] as previously mentioned, the disulfide bond is relatively stable in oxidizing and physiologic pH conditions. Disulfide cleavage-based compounds have been employed for chemosensing, in the development of prodrugs, hydrogels, and nanocarriers, and in material fabrication.[42,43] Thiol-click reaction with disulfides for site-specific protein−polymer conjugation has been reported previously, for instance, the generation of free thiols by reducing the cystine disulfide bridge in a peptide (salmon calcitonin),[44] or protein (mucin),[45] for subsequent polymer attachment. However, the concept of disulfide-based click reaction with synthetic polymers containing cyclic disulfide bonds has not been reported to our knowledge.

The thermal properties of the synthesized PEUU-SS polymers were determined by DSC with typical curves shown in Figure 2. The PEUU-SS(BDI)1 polymer exhibited an obvious T_m at 20 °C, attributed to melting of the soft segment PCL crystals. This T_m was lower than the 40 °C previously reported with PEUU,[8] possibly due to the cyclic structure of O-DTT decreasing soft segment crystallinity and microphase separation. No melting peak was obvious for PEUU-SS(LDI) polymers, a result that could be attributed to the ethyl ester side chain of LDI, further preventing hard segment domain formation and crystallization of soft segments. The observed T_g of both BDI and LDI-based PEUU-SS polymers was around −60 °C, comparable to that for pure PCL macrodiol,[32] indicating that flexibility would be maintained at physiological temperatures. The postulate that LDI-based polymers are more amorphous than BDI-based polymers is further supported by the WXRD data (Figure 3), and differences in the optical clarity of these materials as the PEUU-SS(BDI)1 films appeared visibly more opaque than PEUU-SS(LDI) films. The DSC curves of PEG bulk-modified PEUU-SS(LDI) polymers were similar to the non-bulk-modified polymers in that amorphous behavior was seen. While the conjugated PEG brush in PEUU-

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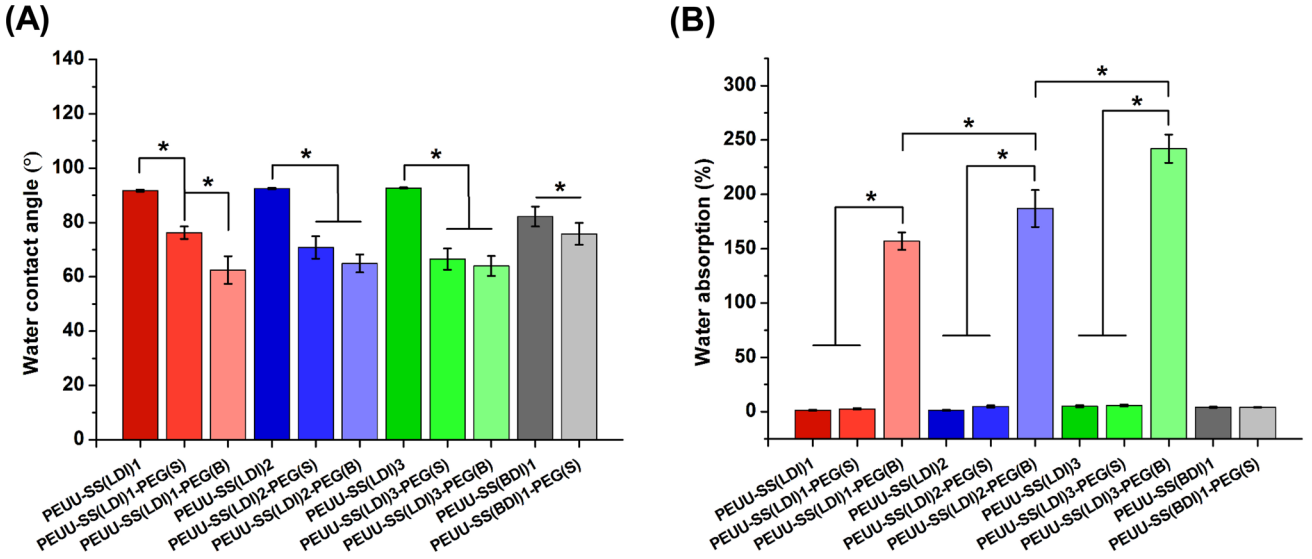

Figure 9. (A) Water contact angle and (B) water absorption of cast films of PEUU-SS polymers, with bulk PEG(B) and surface PEG(S) click modification by PEG acrylate. *$p < 0.05$.

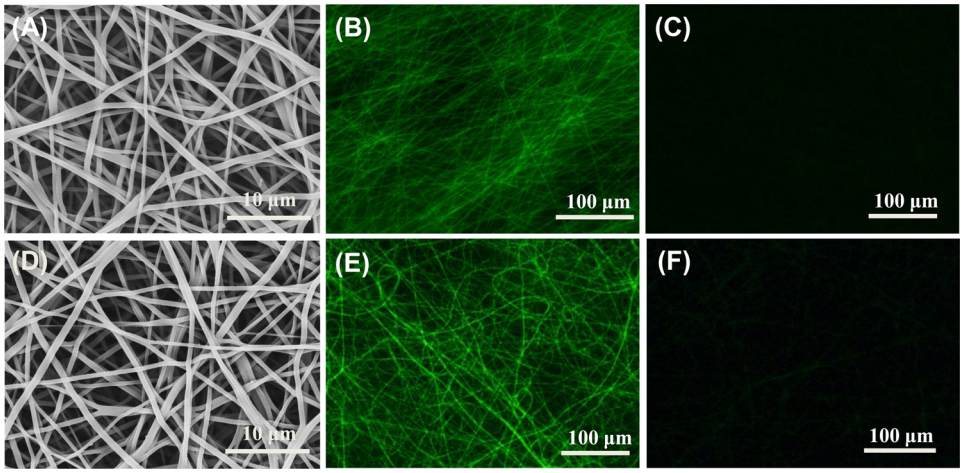

Figure 10. (A) SEM of electrospun PEUU-SS(BDI)1 fibers. (B) Fluorescence images of ES PEUU-SS(BDI)1 fibers labeled with Alexa Fluor 488 C_5 maleimide and (C) control of ES PEUU-SS(BDI)1 fibers after immersion in the Alexa Fluor 488 C_5 maleimide solution. (D) SEM of TPS-maleimide surface click modified ES PEUU-SS(BDI)1 fibers. (E) ES PEUU-SS(BDI)1-TPS(S) fibers labeled with fluorescein-NHS at 4 °C, 24 h and (F) control of non TPS-maleimide surface click modified ES PEUU-SS(BDI)1 fibers immersed in fluorescein-NHS at 4 °C, 24 h.

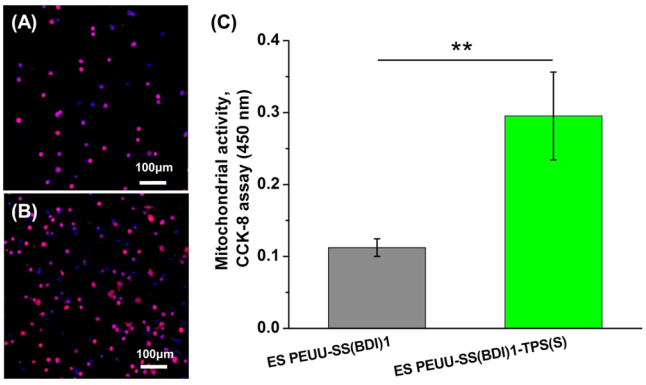

Figure 11. Confocal laser scanning micrographs showing endothelial progenitor cells (EPC) adhesion on ES PEUU-SS(BDI)1 (A) and ES PEUU-SS(BDI)1-TPS(S) (B) surfaces in serum-free medium with DAPI (blue) and phalloidin (red) staining. (C) Short-term adherent EPC metabolic activity ($n = 4$, **$p < 0.01$).

SS(LDI) polymers might be expected to reduce crystallinity, because the unmodified polymers did not show crystalline domains, no clear change was apparent (Figure S5 in the Supporting Information).

The mechanical behavior of scaffolding materials for tissue engineering or temporary mechanical support applications in vivo is an important consideration in material design and selection, with the objective often being to mimic native tissue properties and to provide an appropriate transmission of mechanical load to developing tissue at a cellular level.[46−48] One of the attractive properties of PU elastomers for biomedical applications is their tunable physical properties. The synthesized PEUU-SS polymers demonstrate high distensibility and elasticity, with tensile strength and initial uniaxial tensile moduli that are generally in the range of soft tissue such as human blood vessels.[46] Differences in the initial and 100% modulus as well as the shape of the tensile stress−strain curves between LDI- and BDI-based PEUU-SS polymers might be explained by the previously proposed differences in

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the extent of phase separation and crystallinity in the respective polymers. This effect would be mediated by local steric hindrance from the ethyl ester side chain of LDI in PEUU-SS(LDI) polymers acting to decrease inter- and intramolecular hydrogen bond formation,[49] leading to a significantly lower modulus. This effect may also be reflected in the strain recovery properties of the PEUU-SS polymers after the first cycle in cyclic tensile testing (Figure 5). A small set was seen for all PEUU-SS polymers at 30% deformation, but a much larger set was observed for PEUU-SS(BDI)1 versus PEUU-SS(LDI) at 400% deformation. These results are consistent with a previous report by Ma et al. showing large-strain-induced recrystallization of a BDI-based PEUU with PCL soft segment and less set being seen in poly(ester urethane)ureas with amorphous polyester soft segments,[33] suggesting that a disrupted crystallinity in the PEUU-SS(LDI) polymers would lead to less large-strain-induced crystallization and more strain recovery.

In considering the potential for the PEUU-SS polymers to degrade by hydrolysis or under enzymatic exposure, it was found that degradation was more extensive in lipase solution relative to PBS or elastase solution, presumably due to ester bond sensitivity to lipase.[50,51] Furthermore, PEUU-SS(BDI)1 samples, with a higher degree of crystallinity in the soft segment, degraded significantly faster than PEUU-SS(LDI)1 samples. This result was counter to the common relationship of the degradation rate decreasing with increased crystallinity for polyesters in lipase solution.[52] However, a similar observation was made by Kim et al.,[51] where they synthesized a series of polyester urethanes using 1,6-hexane diisocyanate (HDI), 4,4′-methylene bisphenyl diisocyanate (MDI), and 4,4-methylene biscyclohexyl diisocyanate (HMDI) and PCL as a polyol component. They accessed the degradation of these polymers in lipase and found that the HDI-based polymer was more degradable than MDI- and HMDI-based polymers, even though it had higher phase separation and crystallinity in the soft segment. In a similar study, Tang et al.[53] found the same phenomenon when assessing the enzymatic stability of polycarbonate urethanes derived from these three diisocyanates in cholesterol esterase (CE). In their report, they proposed that an HDI-based polycarbonate urethane had lower hydrogen bonding in the carbonates, making these groups more vulnerable to hydrolysis, and concluded that the extent of CE-catalyzed hydrolytic degradation was highly dependent on the nature of hard segment interactions within the polymer and at the surface. More specifically, the degree of phase separation and soft segment crystallinity was less important in comparison with the hydrogen bonding among the carbonate and urethane linkages. These results correspond to the current finding of higher degradation of BDI-based PEUU versus LDI-based PEUU in lipase solution. This might be due to the relatively stronger hydrogen bonding among the ester and urethane or urea linkages in the LDI-based PEUU. This remains speculative, however.

Aside from considering mechanical signals and degradation when developing smart biomaterials for regenerative medicine or drug delivery purposes, the introduction of biofunctionality by bulk or surface grafting is an important consideration.[13−15,54] Achieving such modifications under mild physiologic conditions is attractive in that it allows a broader consideration of relatively labile biofunctional molecules.[2,7,8] Both bulk and surface click modification in the presence of air and water were thus pursued in this study. BDI-based PEUU-SS polymers are difficult to dissolve in organic solvents, except in DMSO at elevated temperature or HFIP, neither of which is attractive for disulfide reduction,[55] while LDI-based PEUU-SS polymers showed good solubility in DMF, a suitable solvent for thiol−ene reaction. The LDI-based polymers were thus chosen for the bulk click reaction using PEG acrylate as a model-modifying molecule. The results showed reduction of the cyclic disulfide by Bu_3P and modification by PEG acrylate in a one-pot, two-step process with grafting efficiency close to 100%, even in the presence of air. It has reported that P_3R (0.7 to 1 equiv Bu_3P or TCEP), employed for the thio-Michael step, can act as an in situ reducing agent, preventing the reformation of the disulfide bridge, and reducing disulfide groups quickly in the presence of air and water.[44,56] We further found that the modification efficiency could be tuned at the preset values (25, 50, 100%) by controlling the amount of Bu_3P, as determined by ^1H NMR spectroscopy. Furthermore, the existence of air and water showed little effect on the reduction and modification. This should allow for a range of covalent attachment strategies in a fast and controllable manner.

Surface click modification on both LDI- and BDI-based PEUU-SS cast films with PEG acrylate were further performed and characterizations were carried out on films of PEG bulk-modified PEUU-SS(LDI). The FTIR spectra showed the ether peak increase after PEG modification (Figure 8), confirming the bulk and surface click modification of PEUU with disulfide groups. This was also supported by decreased contact angle measurements after PEG click modification on the surface and in the bulk. Water absorption data similarly provided evidence of bulk modification with PEG, with water absorption increasing with more disulfide groups available for bulk click modification with PEG acrylate (Figure 9); however, the increased swellability results in these bulk modified polymers being weak in an aqueous environment, to the point of being water-soluble for PEUU-SS(LDI)10 with PEG modification (data not shown). The strain and strength at break of dry PEG bulk modified PEUU-SS(LDI) polymers were significantly decreased compared with their respective nonmodified polymers (Figure S6 and Table S2 in the Supporting Information), possibly due to the attached PEG affecting hydrogen bond formation in the hard domains. As a result, the polymers that were bulk modified with PEG were considered to be less desirable for application as tissue engineering scaffolds in the physiologic aqueous environment. For the same reason, bulk modification with hydrophilic TPS was not pursued for PEUU-SS (LDI) polymers. Considering the degradation of PEG bulk-modified polymers, as expected, films of these materials degraded much faster than their nonmodified counterparts (Figure S7 in the Supporting Information). This can be attributed to the increased water absorption, promoting ester bond hydrolysis, as has been observed with increasing PEG content in the backbone with other poly(ester urethane)-ureas.[57]

For applicability in biomedical applications, particularly as a tissue scaffold, the processability of the described polymers is an important consideration. An advantage of thermoplastic elastomers is the accessibility of various solvent-based processing methods, such as electrospinning, to easily achieve a porous format that would be applicable in a number of applications. In this study, we focused on a functionalization of PEUU-SS that could be carried out easily and under mild aqueous conditions for potential cardiovascular applications. We have prepared a series of PEUU-SS(LDI) with different

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surface disulfide densities, and all of the developed PEUU-SS(LDI) polymers could be electrospun into fibers; however, with this processing technique the created microfibrous scaffolds experienced shrinkage upon removal from the collection mandrel and upon placement in water, which disturbed the underlying fibrous morphology. This effect was attributed to the amorphous nature of the PEUU-SS(LDI) following the high strain processing technique of electrospinning. For this reason, PEUU-SS(BDI) was selected for use with the electrospinning processing method to create microfibers with stable morphology (after immersion in water or ethanol) for further surface TPS peptide modification. Since Asahara et al.[58] first reported the existence of a bone-marrow-derived circulating progenitor for the endothelial lineage termed EPCs in 1997, there has been interest in capturing EPCs for in situ endothelialization of blood-contacting medical devices. Immobilization of ligands for cell capture such as antibodies, peptides, magnetic molecules, oligosaccharides, and aptamers have been reported.[59] TPS peptide isolated by phage display technology has shown high affinity and specificity to human late-EPCs, as reported by Veleva et al.[31] They further covalently attached this peptide onto methacrylic terpolymer matrices by chain-transfer free radical polymerization and showed that binding affinity was retained in a serum-free medium but eliminated in the presence of serum due to the nonspecific protein adsorption masking the surface.[32] In another report, dual-functionalized PCL film surfaces with TPS and PEG based on host−guest inclusion complexation between β-cyclodextrin (β-CD) and adamantane demonstrated the binding affinity of TPS peptide to EPCs in the presence of serum with antifouling properties provided by PEG.[60] Our results showed that PEUU-SS(BDI)1 was able to be electrospun into a microfibrous format with subsequent surface functionalization by the peptide TPS, using the disulfide-based click reaction. Fluorescence microscopy demonstrated the attachment of the designed TPS-maleimide onto the ES PEUU-SS(BDI)1 fibers. TPS-modified ES PEUU-SS(BDI) fibrous sheets experienced significantly improved EPC adhesion at short times in serum-free medium (Figure 11), suggesting the potential to improve endothelialization in cardiovascular applications, although masking effects from blood proteins have not yet been evaluated; however, it should be possible to click on both an adhesive peptide and PEG, demonstrated independently here, to provide a more specific adhesion signal and minimize nonspecific protein-binding effects.

There are several limitations worth noting in this report. Although, short-term degradation of the PUs with disulfide groups was performed in vitro with PBS, lipase, and elastase solutions and degradation tendencies could be observed and contrasted, the mechanism of enzymatic degradation leading to the observed results remains speculative. Furthermore, the in vivo degradation environment, and particularly the presence of reactive oxygen species and other degradative actors from local macrophages, will almost certainly lead to different degradation behavior, most likely a faster rate.[61] In addition, the effect of surface modification was only studied acutely with the model molecules (PEG and TPS) to demonstrate the feasibility and ease of this modification approach. Furthermore, in-depth study with specific modifying molecules would follow with specific application development. For instance, with the attachment of an EPC-supportive adhesion peptide such as TPS, one would desire to see longer period effects in vitro, in vivo feasibility in cardiovascular settings, and possibly the introduction of a spacer molecule to provide better access for adhesion receptors to the anchored peptide.[62]

5. CONCLUSIONS

In the current study, biodegradable PEUU elastomers with cyclic disulfide groups were developed by the incorporation of O-DTT. Alternative use of LDI and BDI in PU synthesis led to different crystallinity, presumably due to the interactions of the cyclic structure of O-DTT and the side chain of LDI in the hard segment, which affected the thermal and mechanical properties as well as the degradation behavior. PEG acrylate used as a model functionalizing molecule was successfully click attached (bulk and surface) onto PEUU-SS polymers and substrates, and the disulfide modification could be accomplished in a one-pot protocol under mild conditions with rapid reaction rates and high yield. Furthermore, a functional peptide (TPS-maleimide) was attached on the surface of ES PEUU-SS(BDI) fibers by disulfide-based click reaction, and the binding affinity of TPS peptide to EPCs was preserved under serum-free medium, suggesting the potential for this approach in cardiovascular applications. The cyclic disulfide containing biodegradable PUs reported in this work provide an option for cardiovascular and other soft tissue regenerative medicine applications, where a temporary, elastic scaffold with designed biofunctionality from a relatively simple click chemistry approach is desired.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of TPS-maleimide, PEUU-SS polymers or fibers with PEG and TPS modification, as well as evaluation of biocompatibility. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Chen, Q. Z.; Liang, S. L.; Thouas, G. A. *Prog. Polym. Sci.* **2013**, *38*, 584−671.
(2) Liu, Q. Y.; Jiang, L.; Shi, R.; Zhang, L. Q. *Prog. Polym. Sci.* **2012**, *37*, 715−765.
(3) Sobczak, M. *Polym.-Plast. Technol. Eng.* **2015**, *54*, 155−172.
(4) Serrano, M. C.; Chung, E. J.; Ameer, G. A. *Adv. Funct. Mater.* **2010**, *20*, 192−208.
(5) Cherng, J. Y.; Hou, T. Y.; Shih, M. F.; Talsma, H.; Hennink, W. E. *Int. J. Phytorem.* **2013**, *450*, 145−162.

DOI: 10.1021/acs.biomac.5b00192
Biomacromolecules 2015, 16, 1622−1633

(6) Irimia-Vladu, M. *Chem. Soc. Rev.* **2014**, *43*, 588−610.
(7) Fang, J.; Ye, S. H.; Shankarraman, V.; Huang, Y. X.; Mo, X. M.; Wagner, W. R. *Acta Biomater.* **2014**, *10*, 4639−4649.
(8) Hong, Y.; Ye, S. H.; Pelinescu, A. L.; Wagner, W. R. *Biomacromolecules* **2012**, *13*, 3686−3694.
(9) Yang, L. X.; Wei, J. Z.; Yan, L. S.; Huang, Y. B.; Jing, X. B. *Biomacromolecules* **2011**, *12*, 2032−2038.
(10) Ott, C.; Easton, C. D.; Gengenbach, T. R.; McArthur, S. L.; Gunatillake, P. A. *Polym. Chem.* **2011**, *2*, 2782−2784.
(11) Basko, M.; Bednarek, M.; Nguyen, L. T. T.; Kubisa, P.; Du Prez, F. *Eur. Polym. J.* **2013**, *49*, 3573−3581.
(12) Fournier, D.; Du Prez, F. *Macromolecules* **2008**, *41*, 4622−4630.
(13) Jun, H. W.; Taite, L. J.; West, J. L. *Biomacromolecules* **2005**, *6*, 838−844.
(14) Stachelek, S. J.; Finley, M. J.; Alferiev, I. S.; Wang, F. X.; Tsai, R. K.; Eckells, E. C.; Tomczyk, N.; Connolly, J. M.; Discher, D. E.; Eckmann, D. M.; Levy, R. J. *Biomaterials* **2011**, *32*, 4317−4326.
(15) Morral-Ruiz, G.; Melgar-Lesmes, P.; Solans, C.; Garcia-Celma, M. J. *J. Controlled Release* **2013**, *171*, 163−171.
(16) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004−2021.
(17) Lutz, J. F. *Angew. Chem., Int. Ed.* **2007**, *46*, 1018−1025.
(18) Such, G. K.; Johnston, A. P. R.; Liang, K.; Caruso, F. *Prog. Polym. Sci.* **2012**, *37*, 985−1003.
(19) Golas, P. L.; Matyjaszewski, K. *Chem. Soc. Rev.* **2010**, *39*, 1338−1354.
(20) Coneski, P. N.; Schoenfisch, M. H. *Polym. Chem.* **2011**, *2*, 906−913.
(21) Hrsic, E.; Keul, H.; Möller, M. *Eur. Polym. J.* **2012**, *48*, 761−768.
(22) Tanaka, A.; Kohri, M.; Takiguchi, T.; Kato, M.; Matsumura, S. *Polym. Degrad. Stab.* **2012**, *97*, 1415−1422.
(23) Masuko, T.; Minami, A.; Iwasaki, N.; Majima, T.; Nishimura, S. I.; Lee, Y. C. *Biomacromolecules* **2005**, *6*, 880−884.
(24) Aoki, D.; Teramoto, Y.; Nishio, Y. *Biomacromolecules* **2007**, *8*, 3749−3757.
(25) Keul, H.; Mommer, S.; Möller, M. *Eur. Polym. J.* **2013**, *49*, 853−864.
(26) Lowe, A. B. *Polym. Chem.* **2010**, *1*, 17−36.
(27) Chen, S. Y.; Smith, L. M. *Langmuir* **2009**, *25*, 12275−12282.
(28) Goldmann, A. S.; Barner, L.; Kaupp, M.; Vogt, A. P.; Barner-Kowollik, C. *Prog. Polym. Sci.* **2012**, *37*, 975−984.
(29) Li, Y. M.; Yang, M. Y.; Huang, Y. C.; Song, X. D.; Liu, L.; Chen, P. R. *Chem. Sci.* **2012**, *3*, 2766−2770.
(30) Calderon, M.; Graeser, R.; Kratz, F.; Haag, R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3725−3728.
(31) Veleva, A. N.; Cooper, S. L.; Patterson, C. *Biotechnol. Bioeng.* **2007**, *98*, 306−312.
(32) Veleva, A. N.; Heath, D. E.; Cooper, S. L.; Patterson, C. *Biomaterials* **2008**, *29*, 3656−3661.
(33) Ma, Z. W.; Hong, Y.; Nelson, D. M.; Pichamuthu, J. E.; Leeson, C. E.; Wagner, W. R. *Biomacromolecules* **2011**, *12*, 3265−3274.
(34) Ravi, S.; Krishnamurthy, V. R.; Caves, J. M.; Haller, C. A.; Chaikof, E. L. *Acta Biomater.* **2012**, *8*, 627−635.
(35) Bravo-Osuna, I.; Teutonico, D.; Arpicco, S.; Vauthier, C.; Ponchel, G. *Int. J. Pharm.* **2007**, *340*, 173−181.
(36) Zhao, T.; Li, J. A.; Chen, A. F. *Am. J. Physiol.: Endocrinol. Metab.* **2010**, *299*, 110−116.
(37) Wong, S. C.; Baji, A.; Leng, S. W. *Polymer* **2008**, *49*, 4713−4722.
(38) Wong, L. J.; Sevimli, S.; Zareie, H. M.; Davis, T. P.; Bulmus, V. *Macromolecules* **2010**, *43*, 5365−5375.
(39) Massia, S. P.; Hubbell, J. A. *Ann. N.Y. Acad. Sci.* **1990**, *589*, 261−270.
(40) Hashizume, R.; Hong, Y.; Takanari, K.; Fujimoto, K. L.; Tobita, K.; Wagner, W. R. *Biomaterials* **2013**, *34*, 7353−7363.
(41) Desroches, M.; Caillol, S.; Lapinte, V.; Auvergne, R.; Boutevin, B. *Macromolecules* **2011**, *44*, 2489−2500.
(42) Lee, M. H.; Yang, Z.; Lim, C. W.; Lee, Y. H.; Dongbang, S.; Kang, C.; Kim, J. S. *Chem. Rev.* **2013**, *113*, 5071−5109.
(43) Yu, S. J.; Ding, J. X.; He, C. L.; Cao, Y.; Xu, W. G.; Chen, X. S. *Adv. Healthcare Mater.* **2014**, *5*, 752−760.
(44) Jones, M. W.; Mantovani, G.; Ryan, S. M.; Wang, X. X.; Brayden, D. J.; Haddleton, D. M. *Chem. Commun.* **2009**, 5272−5274.
(45) Tachaprutinun, A.; Pan-In, P.; Samutprasert, P.; Banlunara, W.; Chaichanawongsaroj, N.; Wanichwecharungruang, S. *Biomacromolecules* **2014**, *15*, 4239−4248.
(46) Perez, R. A.; Won, J. E.; Knowles, J. C.; Kim, H. W. *Adv. Drug Delivery Rev.* **2013**, *65*, 471−496.
(47) Murphy, W. L.; McDevitt, T. C.; Engler, A. J. *Nat. Mater.* **2014**, *13*, 547−557.
(48) Stella, J. A.; D'Amore, A.; Wagner, W. R.; Sacks, M. S. *Acta Biomater.* **2010**, *6*, 2365−2381.
(49) Reddy, T. T.; Kano, A.; Maruyama, A.; Takahara, A. *J. Biomater. Sci.* **2010**, *21*, 1483−1502.
(50) Tokiwa, Y.; Suzuki, T. *Nature* **1977**, *270*, 76−78.
(51) Kim, Y. D.; Kim, S. C. *Polym. Degrad. Stab.* **1998**, *62*, 343−352.
(52) Tokiwa, Y.; Calabia, B. P.; Ugwu, C. U.; Aiba, S. *Int. J. Mol. Sci.* **2009**, *10*, 3722−3742.
(53) Tang, Y. W.; Labow, R. S.; Santerre, J. P. *J. Biomed. Mater. Res., Part A* **2001**, *57*, 597−611.
(54) Lutolf, M. P.; Hubbell, J. A. *Nat. Biotechnol.* **2005**, *23*, 47−55.
(55) Tam, J. P.; Wu, C. R.; Liu, W.; Zhang, J. W. *J. Am. Chem. Soc.* **1991**, *113*, 6657−6662.
(56) Burns, J. A.; Butler, J. C.; Moran, J.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 2648−2650.
(57) Guan, J.; Sacks, M. S.; Beckman, E. J.; Wagner, W. R. *Biomaterials* **2004**, *25*, 85−96.
(58) Asahara, T.; Murohara, T.; Sullivan, A.; Silver, M.; vanderZee, R.; Li, T.; Witzenbichler, B.; Schatteman, G.; Isner, J. M. *Science* **1997**, *275*, 964−967.
(59) Avci-Adali, M.; Ziemer, G.; Wendel, H. P. *Biotechnol. Adv.* **2010**, *28*, 119−129.
(60) Ji, Q.; Zhang, S.; Zhang, J. M.; Wang, Z. H.; Wang, J. N.; Cui, Y.; Pang, L. Y.; Wang, S. F.; Kong, D. L.; Zhao, Q. *Biomacromolecules* **2013**, *14*, 4099−4107.
(61) Hafeman, A. E.; Zienkiewicz, K. J.; Zachman, A. L.; Sung, H. J.; Nanney, L. B.; Davidson, J. M.; Guelcher, S. A. *Biomaterials* **2011**, *32*, 419−429.
(62) Beer, J. H.; Springer, K. T.; Coller, B. S. *Blood* **1992**, *79*, 117−128.

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