Air-spun PLA nanofibers modified with reductively-sheddable hydrophilic surfaces for vascular tissue engineering: Synthesis and surface modification

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ABSTRACT
Polylactide is a class of promising biomaterials that hold a great promise for various biological and biomedical applications, particularly, vascular tissue engineering as fibrous mesh to coat inside vascular prosthesis. However, their hydrophobic surface to non-specific interactions and limited ability to further modification are challenging. Here, we report the development of new air-spun PLA nanofibers modified with hydrophilic surfaces exhibiting reduction response. Surface-initiated atom transfer radical polymerization allows for grafting pendant oligo(ethylene oxide)-containing polymethacrylate (POEOMA) from PLA air-spun fibers labeled with disulfide linkages. The resulting POEOMA-g-PLA fibers exhibit enhanced thermal stability and improved surface properties, as well as thiol-responsive shedding of hydrophilic POEOMA by the cleavage of disulfide linkages in response to reductive reactions, thus tuning the surface properties.

1   INTRODUCTION

Polylactide (PLA), along with polycaprolactone and polyglycolide, is a class of hydrophobic aliphatic polyesters based on hydroxyalkanoic acids [1]. These polyesters are biocompatible, biodegradable by enzymatic reactions or hydrolysis in physiological conditions, and used in FDA-approved medical devices. They also exhibit low immunogenicity and good mechanical properties. Being facilitated with these biological properties, PLA and their copolymers are processed to various formats [2]; a promising format is nanofibers fabricated by electrospinning or air-spinning, efficient processing methods to manufacture long fiber structures. Air-spinning is an alternative process to

classical electrospinning, based on stretching of polymer solutions with a high-speed air flow. These fibrous materials possess large surface areas, high porosity, and interconnected network structures. These unique properties have promoted the use of PLA-fibers as useful biomaterials as sutures, implants for bone fixation [3], drug delivery vehicles [4], and tissue engineering scaffolds [5]. Recently, air-spun PLA-fibers have found their applications as biomaterials to coat luminal wall of commercial vascular grafts in vascular surgery.

Vascular grafts are used to replace, bypass, or maintain function of damaged, occluded or diseased blood vessels in small, medium and large diameter. Poly(ethylene terephthalate) (PET) is one of the commercially-available materials for textile vascular prostheses. PET is usually coated with proteins such as gelatin or collagen to prevent blood loss through prosthesis wall [6]. A drawback for the impregnation of proteins involves a dramatic decrease in patency rate with smaller diameters [7]. The use of PLA-based nanofibrous mesh enable to circumvent the problem as well as promote good endothelial cell proliferation in monolayers on the PLA-fiber mesh [8]. Moreover, surface properties (roughness, hydrophilicity, low fouling towards biological molecules) are directly related to immune response and blood coagulation activation [9]. Despite these advances, however, several challenges for the use of conventional PLA-fiber mesh to be addressed remain. One challenge is their hydrophobic surface causing non-specific interactions with proteins in the blood. In this context, a current strategy consists of increasing PLA hydrophilicity, therefore leading to a better resistance against biomolecule fouling and improving hemocompatibility. Another challenge involves their limited ability to further conjugation or modification of their surfaces due to their lack of functionalities. Surface modification of PLA nanofibrous materials with chemical functions, desirably hydrophilicity, or biomolecules could strongly enhance their applicability toward the successful vascular tissue engineering applications.

Several approaches to surface modification of nanofibrous materials have been reported. Click-type orthogonal reactions have been used for enhanced specific protein adhesion [10] and anti-fouling properties [11]. Surface-initiated (SI) atom transfer radical polymerization (ATRP) [12] has also been explored. The SI-ATRP technique allowed for adjusting the surface properties of fibrous materials of polystyrene, PET, PLA, and cellulose. This "grafting from" method was conducted directly from bromine-terminated polystyrene fibers in aqueous solution [13]. However, for most of the fibrous materials, post-immobilization of small molecular weight ATRP initiating species into as-synthesized fibers through either physical absorption [14] or covalent attachment is required prior to SI-ATRP [15].

Stimuli-responsive degradation (SRD) is a dynamic and powerful platform that involves the cleavage of covalent bonds in response to external stimuli [16]. Particularly, disulfides are cleaved to the corresponding thiols in response to reductive reactions [17], enabling enhanced release,[18] changing morphologies [19], or tuning lower critical solution temperature [20]. This unique disulfide-thiol chemistry has been explored to develop a variety of reductively-responsive degradable nanomaterials desirable for various biomedical applications. Typical examples include self-assembled micellar nanocarriers [21], nanocapsules [22], nanogels [23], hydrogels [24], and bioconjugates [25]. Further, PLA fibers modified with sheddable hydrophilic polymers that can undergo reductively-responsive cleavage would be interesting as smart coating materials in constructing PET-based vascular prostheses with tunable surfaces of hydrophobicity and hydrophilicity.

Herein, we report new air-spun PLA nanofibers whose surfaces are modified with reductively-sheddable hydrophilic surfaces as versatile coating biomaterials for vascular surgery. Scheme 1 illustrates our approach to synthesis and surface modification of reductive-responsive PLA fibers labeled with disulfide linkages. High molecular weight PLA-ss-Br homopolymers were first synthesized by ring opening polymerization (ROP) in the presence of a double-head initiator labeled with a disulfide (HO-ss-Br) and then processed to PLA-ss-Br fibers by air-spinning. As a proof-of-concept approach, pendant oligo(ethylene glycol)-containing polymethacrylate (POEOMA) was targeted to modify the surfaces of air-spun PLA fibers using a direct SI-ATRP to be hydrophilic.

POEOMA is an analog of poly(ethylene oxide) (PEO); PEO is biocompatible material that has been FDA-approved for clinical use, has low toxicity, and prevents nonspecific protein adsorption.[26] The successful grafting of POEOMA from PLA fibers enabled improved thermal stability and surface properties. Further, the reductive cleavage of disulfide linages resulted in shedding hydrophilic POEOMA from POEOMA-g-PLA fibers, adjusting their surface properties.
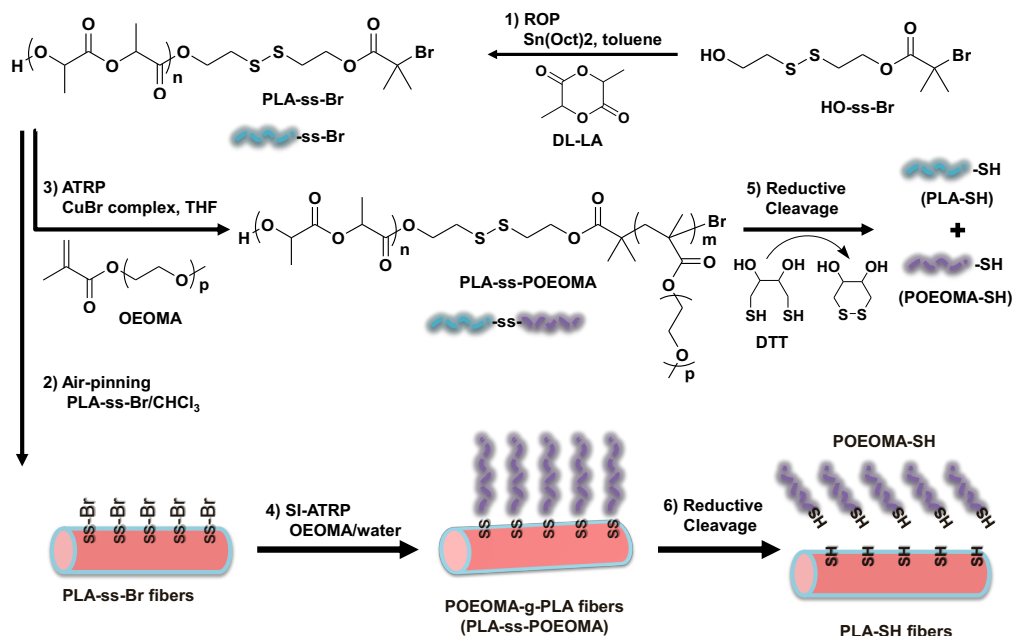
Scheme 1. Illustration of our approach to synthesize reductively-sheddable POEOMA-g-PLA fibers based on PLA-ss-POEOMA block copolymers having disulfides at block junctions using surface-initiated atom transfer radical polymerization of OEOMA in the presence of PLA-ss-Br fibrous macroinitiators and their degradation in response to reductive reactions.

2   RESULTS AND DISCUSSION

The synthesis of HO-ss-Br double-head initiator for both ROP and ATRP is described in our previous publication [27]. In the presence of HO-ss-Br, a series of ROP of D,L-lactide (LA) mediated with tin(II) 2-ethylhexanonate (Sn(Oct)$_2$) in toluene at 120°C was conducted. The results are summarized in Table S1, where the samples are denoted as PLA-x (x: serial number). First, ROP with the targeting degree of polymerization (DP) = 70 defined as the initial mole ratio of [LA]$_0$/[HO-ss-Br]$_0$ = 70/1 yielded PLA-ss-Br homopolymer (PLA-1) with relatively low molecular weight, the number average molecular weight (Mn) = 27.6 kg/mol and narrow molecular weight distribution, Mw/Mn = 1.06. As seen in Figure S1, its gel permeation chromatography (GPC) trace shows a shoulder in high molecular weight region. Peak analysis using a deconvolution method suggests the content of high molecular weight species (HMS) to be 16%. The formation of HMS could be attributed to undesirable side reactions [28]. In an effort to synthesis of high molecular weight PLA-ss-Br with less HMS content, the important parameters such as targeting DP and [Sn(Oct)$_2$]$_0$/[HO-ss-Br]$_0$ ratio were varied. When the targeting DP increased to 500 and 1000, relatively high molecular weight PLA-ss-Br homopolymers with Mn = 65 – 75 kg/mol were obtained with longer polymerization time. However, the undesirable HMS content also increased up to 41%. When the amount of Sn(Oct)$_2$ increased, polymerization was fast, and the HMS contents also increased (PLA-7 and PLA-8 with targeting DP = 1000) (Figure S2). Further increase in targeting DP = 2000 with [Sn(Oct)$_2$]$_0$/[HO-ss-Br]$_0$ = 0.14/1 yielded high molecular weight PLA-9 with Mn = 75.6 kg/mol and HMS = 15%. These

results suggest that the balance of targeting DP, polymerization time, and Sn(Oct)$_2$ content is required for the synthesis of high molecular weight PLA-ss-Br with less HMS content.

Next, air-spinning of the PLA-ss-Br homopolymers dissolved in chloroform was examined. Chloroform is a good solvent to PLA, volatile, and advantageous for spinning purposes. Three homopolymers with different molecular weights, but HMS content < 20%, were selected: PLA-1 (Mn = 27.6 kg/mol), PLA-6 (Mn = 35 kg/mol), and PLA-9 (Mn = 75.6 kg/mol) (see their GPC traces in Figure S1). PLA-1 and PLA-6 with relatively low molecular weights (Mn < 40 kg/mol) were not well air-spun, even at as high as 70% concentration. Scanning electron microscopy (SEM) images in Figure S3 show the presence of some fibers, but mostly large spheres. Such poor spinnability could be attributed to relatively low molecular weight PLAs, and thus lower solution viscosities even at higher concentrations. Promisingly, PLA-9 with Mn >70 kg/mol was fabricated through entanglement of PLA chains to fibrous woven at 30% concentration (Figure 1A and 1C). The average diameter was estimated to be 495 ± 240 nm from approximately 150 fibers (Figure S4).
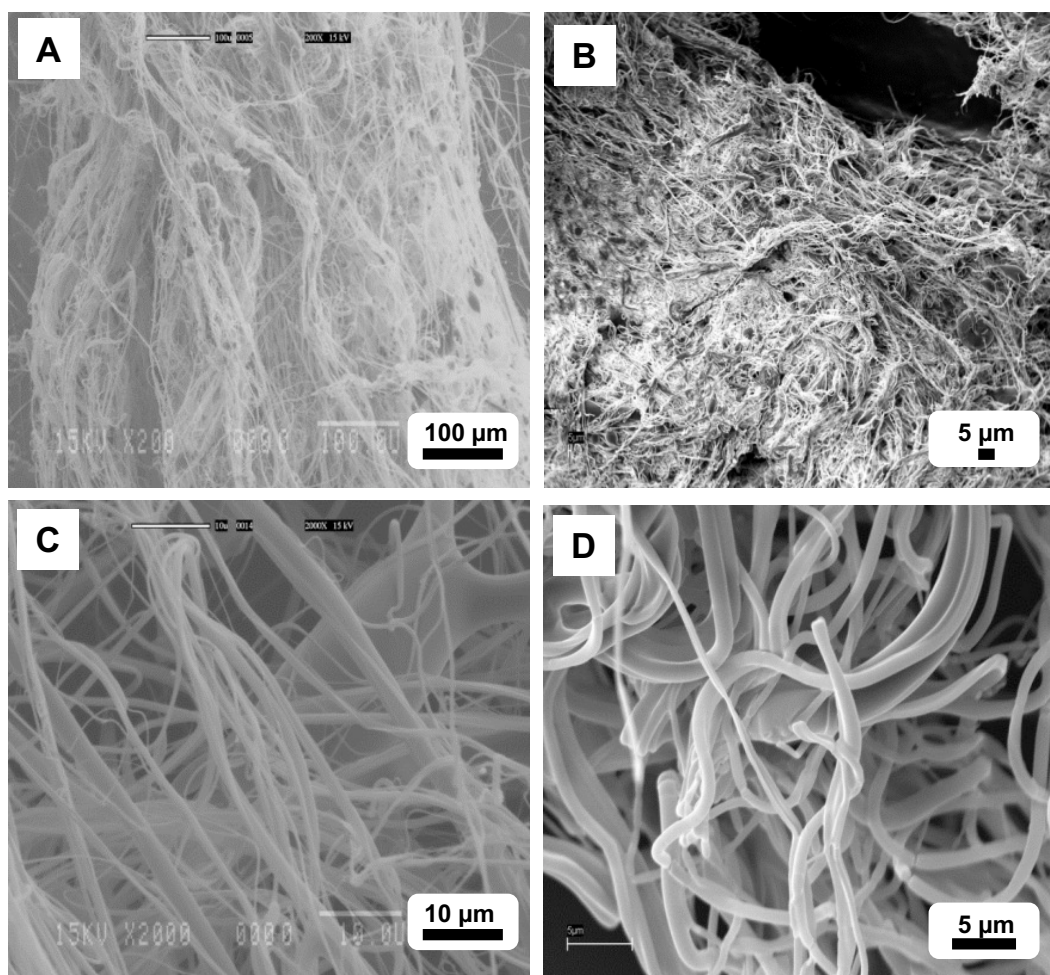

Figure 1. SEM images of PLA-9 fibers (Mn = 75.6 kg/mol) (A, C) and POEOMA-g-PLA BCP-3/fibers (B, D) with lower (A, B) and higher (C, D) magnitudes.

The resulting PLA-ss-Br fibers consist of terminal Br groups. In the presence of PLA-ss-Br fibers, SI-ATRP of OEOMA was investigated to synthesize POEOMA grafted from PLA fibers (POEOMA-g-PLA fibers) based on PLA-ss-POEOMA block copolymers (BCPs) consisting of disulfide linkages at the junctions of PLA and POEOMA blocks. Table 1 summarizes the results. To see the availability of terminal Br groups in PLA-ss-Br homopolymer fibers, their chain extension was first examined

with OEOMA300 with MW = 300 g/mol (BCP-1) and OEOMA950 with MW = 950 g/mol (BCP-2) in homogeneous solution. Aliquots of the dried fibers, OEOMA, and CuBr complex were dissolved in tetrahydrofuran under direct ATRP conditions for 2 hrs. Conversion was determined to be 0.14 for BCP-1 and 0.07 for BCP-2. After purification, their 1H-NMR spectra show typical peaks at 5.0-5.2 ppm (a) corresponding to methine protons in PLA and 0.9-1.0 ppm (b) to backbone methyl protons in POEOMA (Figure 2B for BCP-1 and Figure S5 for BCP-2). From the integral ratio of these peaks [(b/3)/(a/2)], the weight ratio of POEOMA/PLA in BCPs was calculated to be 0.22/1 for BCP-1 and 0.42/1 for BCP-2. These values are close to those calculated using the wt ratio of OEOMA/PLA from the recipe and the determined conversion (Table 1). GPC traces of the purified BCPs evolved to higher molecular weight region. For example, Mn increased from 71.4 kg/mol to 82.0 kg/mol for BCP-1 (Figure S6). These 1H-NMR and GPC results indicate the successful synthesis of PLA-ss-POEOMA BCPs in homogeneous solutions, confirming the presence of terminal Br groups in PLA-ss-Br homopolymer fibers.

 With the promising results, grafting POEOMA from PLA-ss-Br fibers through SI-ATRP in aqueous solution was next examined. Solubility test reveals that PLA fibers remained intact in aqueous solution of OEOMA950, while they were dissolved in aqueous solution of OEOMA300 even at room temperature. Consequently, PLA fibrous mesh was soaked in a solution consisting of OEOMA950, Cu complex, and water for 2 hrs. SI-ATRP was then conducted for 2 h at 30°C, below the glass transition temperature of amorphous PLA ($\approx$ 39°C). The resulting products were purified by extensive dialysis using a dialysis tubing (MWCO = 12 kg/mol) to completely remove unreacted OEOMA950 monomers. 1H-NMR spectrum shows the typical peaks (b) at 0.9-1.0 ppm for POEOMA and 5.0-5.2 ppm (a) for PLA (Figure 2C). The wt ratio of POEOMA/PLA was calculated to be 0.13/1 (Table 1). Further, the GPC trace of the purified BCP slightly evolved to higher molecular weight region (Figure S7). These results suggest the successful grafting POEOMA from PLA fibers, yielding POEOMA-g-PLA fibers (BCP-3/fibers) based on PLA-ss-POEOMA BCPs.

Table 1. Characteristics and molecular weight data of PLA-ss-POEOMA BCPs synthesized by chain extension of PLA-ss-Br fibers with POEOMA under normal ATRP conditions.

Entry	BCP-1	BCP-2	BCP-3/fibers
PLA fiber (mg)	276	208	198
OEOMA/PLA (wt/wt)	1.5/1	4.8/1	4.8/1
OEOMA [M]	0.53	0.50	0.60
CuBr [mM]	5.3	5.0	6.3
OEOMA monomer	OEOMA300	OEOMA950	OEOMA950
Solvent	THF	THF	water
Time (hrs)	6	2	2
Conv [a]	0.14	0.06	NA
POEOMA/PLA (wt/wt),$_{theo}$ [b]	0.21/1	0.35/1	NA
POEOMA/PLA (wt/wt) [c]	0.22/1	0.42/1	0.13/1
M_n (g/mol) [d]	82,000	77,200	67,200
M_w/M_n [d]	1.11	1.14	1.13

a) Determined by 1H-NMR.
b) Estimated by OEOMA/PLA wt ratio x conversion
c) Determined by 1H-NMR with molecular weight of LA and OEOMA
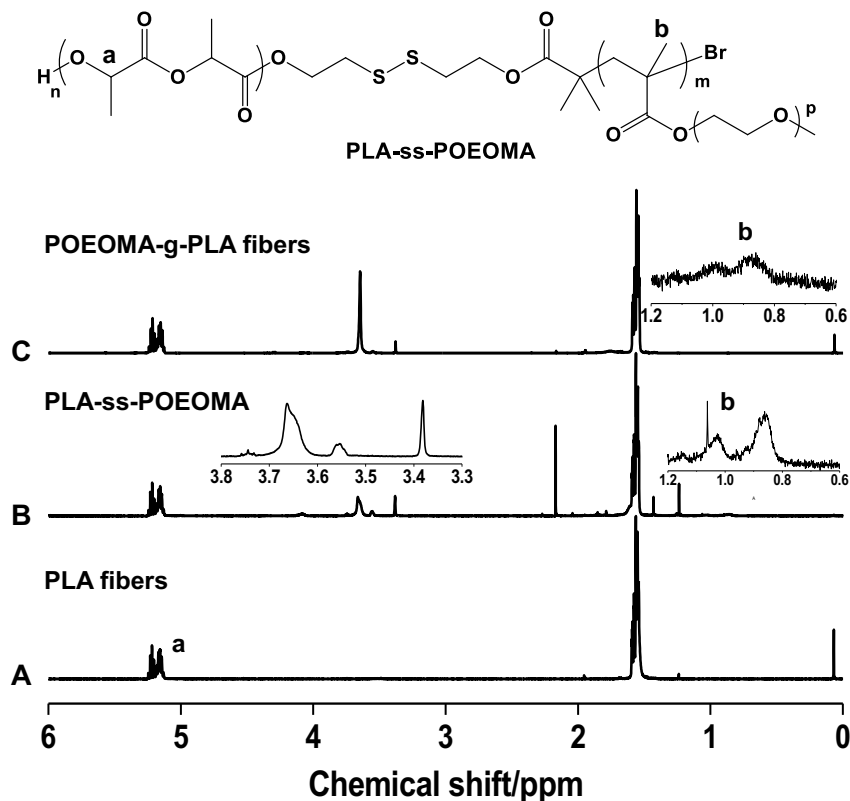d) Determined by GPC with DMF as an eluent.

Figure 2. 1H-NMR spectra of PLA-9 (A), BCP-1 (B), and BCP-3/fibers (C) in CDCl$_3$.

Further, the effect of the grafted POEOMA chains was evaluated on fibrous morphologies by SEM, thermal properties using thermogravimetric analysis (TGA), surface properties using contact angle measurements. SEM images show a relatively dense fibrous form (Figure 1B and 1D). The average diameter of the POEOMA-g-PLA fibers was estimated to be 620 ± 220 nm, larger than pristine PLA fibers by approximately 122 nm (Figure S4). Figure S8 shows TGA data of the weight loss of BCP-3/fibers, compared with PLA-9 fibers and PEO homopolymers as controls, upon heating. For BCP-3/fibers, the major weight loss started at 236°C. This temperature is lower than that (315°C) for PEO homopolymers, but higher than that (215°C) by 20°C for PLA fibers. This result suggests that the tethered POEOMA enhances thermal stability of PLA fibers. Figure 3 shows the results of contact angle measurements with snapshots of water drops on PLA fibrous mesh before and after surface modification with POEOMA. On POEOMA-g-PLA fibers, water drops were immediately absorbed into fibers within 1.5 sec, resulting in sharp decrease in contact angle (Figure 3A). The disappearance of a water drop on BCP fibers was snapshotted in Figure 3B. On hydrophobic PLA homopolymer fibers, however, water drops stayed with a contact angle at 120.5° over time (Figure 3C). This result suggests that the tethered hydrophilic POEOMA increases wettability of hydrophobic PLA fibers, allowing an instantaneous penetration of water, whereas the pristine PLA fibers offered a significant barrier to it. Similar results are reported for other fibrous materials with different chemical structures [10, 14].
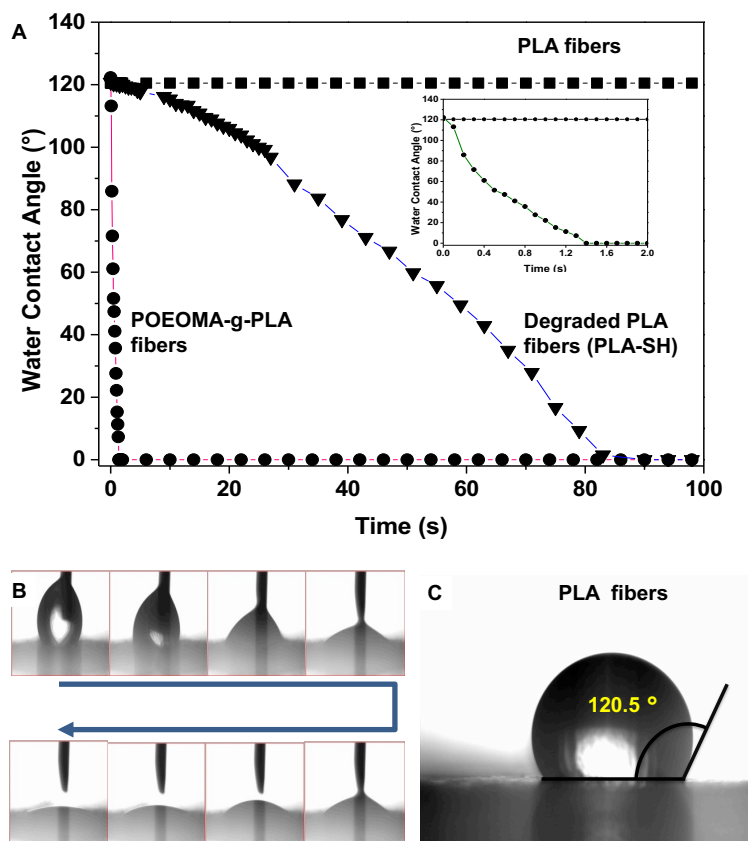
Figure 3. Evolution of contact angle on PLA fibers, POEOMA-g-PLA fibers before and after treatment with DTT (A), and snapshots of water droplets on POEOMA-g-PLA fibers after SI-ATRP (B) and PLA-ss-Br fibers in the pristine state (C). Inset of (A): evolution of contact angle on PLA fibers and POEOMA-g-PLA fibers before DTT treatment.

The POEOMA-g-PLA fibers based on PLA-ss-POEOMA BCPs contain disulfides at block junctions. These disulfide linkages could be cleaved in the presence of DTT, a reducing agent, to the corresponding thiols including POEOMA-SH and PLA-SH as linear polymers or fibers (Scheme 1). GPC results indicate the decrease in molecular weight of PLA-3/fibers from Mn = 71.8 kg/mol to Mn = 67.0 kg/mol in homogeneous DMF solution with excess DTT (Figure S9). 1H-NMR was also used to examine the disulfide cleavage in aqueous solution where BCP-3/fibers exist as meshes. In the presence of excess DTT, the typical peaks at 0.9-1.0 ppm corresponding to backbone methyl protons in POEOMA completely disappeared, while being retained in the absence of DTT (Figure S10). These GPC and NMR results suggest the significant cleavage of disulfide linkages in reducing environments. Such cleavage could result in shedding POEOMA-SH from POEOMA-g-PLA fibers, thus changing the surface polarity of PLA fibers. As seen in Figure 3A, water contact angle on the resulting PLA-SH fibers slowly decreased. After 80 sec, water droplet was completely absorbed in the fibers. Such slow decrease is attributed to the surface of the resulting PLA-SH fibers to be less hydrophilic than POEOMA-g-PLA fibers, but more hydrophilic than pristine PLA fibers.

3   CONCLUSIONS

A new class of air-spun PLA nanofibers modified with thiol-responsive sheddable POEOMA was synthesized by a combination of ROP, air-spinning, and SI-ATRP techniques. High molecular weight PLA-ss-Br with less HMS contents (<15%) ensured good spinnability, yielding PLA fibers functionalized with terminal Br groups at interfaces. SI-ATRP allowed for the modification of the PLA

fibers with tethered hydrophilic POEOMA blocks. The resulting POEOMA-g-PLA fibers with hydrophilic surfaces exhibit improved thermal stability and surface properties such as water content and wetting behavior, confirmed by SEM, TGA, and contact angle measurements. Moreover, shedding hydrophilic POEOMA from POEOMA-g-PLA fibers by the cleavage of disulfide linkages in response to reductive reactions enabled tuning the surface properties. Toward vascular tissue engineering, the controlled and enhanced release of therapeutics upon the cleavage of disulfide linkages in response to reductive reactions [29] of the new POEOMA-g-PLA fibrous mesh as well as by varying hydrophobicity driven by their nanostructures fabricated on PET films [30] is currently under investigation.

ACKNOWLEDGEMENTS

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