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Article

Fabrication of Bioactive Surfaces by Functionalization of Electroactive and Surface-Active Block Copolymers

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Abstract: Biofunctional block copolymers are becoming increasingly attractive materials as active components in biosensors and other nanoscale electronic devices. We have described two different classes of block copolymers with biofuctional properties. Biofunctionality for block copolymers is achieved through functionalization with appropriate biospecific ligands. We have synthesized block copolymers of electroactive poly(3-decylthiophene) and 2-hydroxyethyl methacrylate by atom transfer radical polymerization. The block copolymers were functionalized with the dinitrophenyl (DNP) groups, which are capable of binding to Immunoglobulin E (IgE) on cell surfaces. The block copolymers were shown to be redox active. Additionally, the triblock copolymer of α, ω-bi-biotin (poly(ethylene oxide)-b-poly (styrene)-b-poly(ethylene oxide)) was also synthesized to study their capacity to bind fluorescently tagged avidin. The surface-active property of the poly(ethylene oxide) block improved the availability of the biotin functional groups on the polymer surfaces. Fluorescence microscopy observations confirm the specific binding of biotin with avidin.

Keywords: block copolymers; biosensor; ionic polymerization; atom transfer radical polymerization; fluorescence microscope

1. Introduction

Polymeric materials functionalized with appropriate biospecific ligands have been proved to be effective in targeted drug-delivery and bio-sensing applications, to name a just few examples [1,2]. Nano-structured polymeric materials are usually more effective carriers of therapeutic agents, especially in *in vivo* applications [3]. Some of these architectures include nanofibers (obtained by self-assembly of the polymers or by electrospinning) [4], nanoparticles (nanospheres or nanorods) [5], nanoplates [6], nanoribbons [7], *etc*. Giant macromolecules or polymers are very robust in terms of applications, as they are found in virtually all areas of daily life. A notable attribute of some polymers is, in fact, that they can easily undergo modification to mimic biomolecules [8].

Biofunctional polymers have been prepared with wide variety of applications ranging from diagnostics to therapeutics and tissue engineering [9–13]. An approach to synthesizing polymers with bioactive properties is directly attaching a bioactive moiety to the synthetic polymer. An example of this is the covalent attachment of a short peptide chain to the polymer, as reported by Baird *et al*. [14]. Another approach is the synthesis of polymers containing species capable of specific ligand-receptor interactions. This is essentially conjugating a polymer with a ligand or functional groups that antibodies recognize and adhere to [15]. A method of achieving this has been reported by Sannigrahi *et al*., whereby the ligand, 2,4-dinitrophenyl (DNP), recognized by the antibody, Immunoglobulin E (IgE), was incorporated into a block copolymer [16]. Multivalent antigen-antibody interactions have paved the way for the development of many ligand-targeted drug delivery vehicles to treat a number of diseases [17,18]. The use of the multivalent binding effect has been demonstrated to maximize targeting efficiency in linear-dendritic block copolymer micelles [19]. The interaction of biotin and avidin as a ligand-receptor pair is commonly used in the field of biology and medicine for purification, localization and diagnostics, serving as a well-defined model system to probe bioavailability [20].

Surface accessibility of ligands is crucial to receptors, whether in drug delivery systems or diagnostics. Antigen-antibody characteristic of DNP-IgE has been tested in our group with a synthesized block copolymers α,ω-bi(2,4-dinitrophenyl caproic) (poly(ethyleneoxide)-b-poly(2-methoxystyrene)-b-poly(ethylene oxide)) [16,21]. Block copolymers contains at least two polymer blocks which phase separate into two or more immiscible phase domains within the polymeric region. Moreover, a bioactive species is covalently attached with one of the polymer blocks. By selectively attaching bioactive species to at least one polymer block, self-assembled clusters of bioactive species are created. Poly(ethylene oxide) has added advantages of surface activity if it is part of block copolymer. Electroactive block of copolymers [22] or hybrid hydrogel [23,24] could introduce conductivity which could essentially develop variety of applications such as biosensors, electrostimulated drug release devices, *etc*. Cytocompatible poly(2-hydroxy ethyl methacrylate) as a part of block copolymer could enhance the attachment and growth of various dependent cells [25]. Therefore, it could be assumed that the use of block copolymers may be an effective approach to fabricate thin films in order to explore their biological properties.

In order to test our hypothesis that functional block copolymers are most suitable for fabricating a surface coated with a bioactive group, we have synthesized two sets of functional block copolymers and fabricated surface from the block copolymers. The triblock copolymers include α, ω-bi-biotin (poly(ethylene oxide)-b-poly(styrene)-b-poly(ethylene oxide)) (Biotin-PEO-PS-PEO-Biotin) and block copolymers from 3-decylthiophene and 2-hydroxyethyl methacrylate into which DNP groups (DNP-PHEMA-P3DT) were incorporated. The 3-decylthiophene block, is the electroactive portion of the copolymer due to its inherent, conductive nature. The functional copolymers were then processed into structures decorated with either the biotin or the DNP functional groups.

2. Experimental Section

2.1. Chemicals and Reagents

Potassium metal, calcium hydride, benzophenone, styrene, ethylene oxide, dicyclohexylcarbodimide (DCC), dimethylaminopyridine (DMAP) and dodecanethiol AuNPs were procured from Aldrich Chemical Company, Milwaukee, WI, USA. Monomers were dried over CaH_2 and distilled prior to polymerization. Tetrahydrofuran (Fisher Scientific, Pittsburgh, PA, USA) was purified by refluxing over fresh sodium benzophenone complex. 3-Decylthiophene, 2-hydroxyethyl methacrylate, lithium aluminum hydride, $LiAlH_4$ (1.0 M in THF), ethyl magnesium bromide (3 M in diethyl ether), phosphorous oxychloride, 2-bromopropionyl bromide, triethylamine, N-methylformamide, anhydrous magnesium sulfate, N-bromosuccinimide, and all common organic solvents used were obtained from Sigma-Aldrich Co. Anhydrous sodium acetate was purchased from Fisher Chemical Co. The IgE solution used was provided by the Baird-Holowka Lab. at Cornell University, Ithaca, NY, USA.

2.2. Preparation of Difunctional Initiator

The di-carbanion initiator (α-methylstyrene dimer di-anion, in Scheme 1) used for the polymer synthesis was prepared by reacting potassium mirror (5 g) with α-methylstyrene (6 mL) in 200 mL THF. Under high vacuum techniques the solution turned red after shaking, indicating the formation of the di-initiator. The di-initiator was later stored under high vacuum in ampoules.

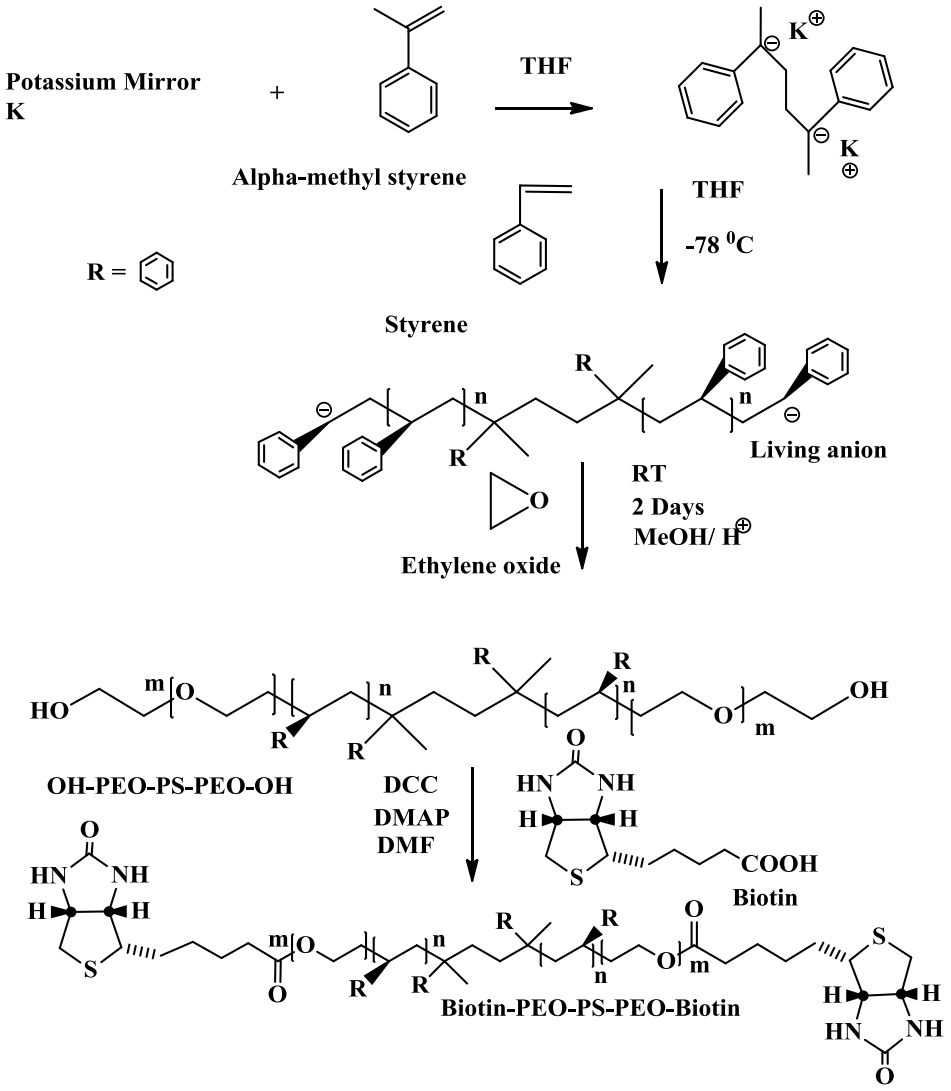
2.3. Synthesis of α, ω-bi-Biotin(Poly(Ethylene Oxide)-b-Poly(Styrene)-b-Poly(Ethylene Oxide)), (Biotin-PEO-PS-PEO-Biotin)

Through an electron transfer reaction of α-methylstyrene and potassium (mirror) metal the di-initiator was created [16]. Using standard high vacuum techniques; polymerization was carried out in all-glass sealed reactors. The reactors were cleaned, annealed; and later equipped with monomers (styrene and ethylene oxide) and initiator ampoules (with break-seals). The reactor was attached to the vacuum line, and 10 mL THF (~10/1 solvent/monomer, vol/ vol) was distilled into the flask. Through heat-sealing with a hand torch technique, the reactor was later detached from the vacuum line and transferred to a dry ice bath maintained at −78 ℃. First, 4 mL (0.27 mmol/ mL) initiator was added by breaking the break-seal of the initiator ampoule. This was then followed by the introduction of styrene (3 mL). There was a color change of the solution from light red to deep red, indicating the formation of polystyrene anion. Ethylene oxide (1 mL) was added to the solution after the reaction was allowed to

proceed for 40 min. The color disappeared completely after a few minutes signifying the initiation of the second monomer. For two days at room temperature polymerization of the second monomer (ethylene oxide) was allowed to ensure completion before terminating with the addition of methanol and hydrochloric acid to produce the α, ω-dihydroxyl polymer (HO-PEO-PS-PEO-OH). The functional polymers were purified by precipitation into hexanes. Yields were >90%.

Reaction of the α,ω-dihydroxyl polymer with biotin in the presence of *N*, *N*′-dicyclohexylcarbodimide (DCC) and dimethylaminopyridine (DMAP) resulted in the formation of *α*, *ω-bi*-biotin (poly(ethylene oxide)-b-poly(styrene)-b-poly(ethylene oxide)) (Biotin-PEO-PS-PEO-Biotin). To purify the product, the excess biotin was removed using a separatory method. Sodium bicarbonate was added to the round bottom flask containing the product forming thick white slurry. The slurry was then transferred to a separatory funnel where methylene chloride was later added. The contents in the flask were then agitated forming a mixture. After allowing the contents to settle for 15 min, two distinct layers formed. The lower layer containing the pure functionalized polymer was extracted leaving behind the top layer. The top layer was comprised of biotin salts which was formed from the excess biotin.

Scheme 1. Synthetic sketch of α, ω-bi-biotin(poly(ethylene oxide)-b-poly(styrene)-b-poly(ethylene oxide)), Biotin-PEO-PS-PEO-Biotin.



2.4. Synthesis of 2,5-Dibromo-3-Decylthiophene

N-bromosuccinimide, NBS was re-crystallized from boiling water, prior to use. The monomer, 3-decylthiophene, was dibrominated according to the method of Iraqi *et al.* [26] as follows: a solution of NBS (5.34 g, 30 mmol) in DMF (100 mL) placed in a pressure equalization addition funnel was added drop-wise in the dark, at a temperature of −20° C to a solution of 3-decylthiophene (3.14 g, 14 mmol) in DMF (100 mL) in a 500 mL round bottom flask under a blanket of nitrogen. When the addition of NBS was over, the mixture was stirred at room temperature overnight after which it was poured over ~600 mL of ice water. Extraction was done in dichloromethane (3 × 100 mL), organic phases combined and subsequently washed in water (5 × 100 mL). After the last round of washing, the mixture was dried over anhydrous magnesium sulfate to remove the water. The mixture was then dried in a vacuum to remove residual solvent and the title compound, 2,5-dibromo-3-decylthiophene was obtained as a yellow oil. The dibrominated monomer was purified by distillation under reduced pressure. Yield ~95%. ^{1}H NMR ($CDCl_3$, TMS) δ (ppm): 0.91–0.94 (t 3H), 1.27–1.75 (m 16 H), 2.66 (t 2H), 6.98.

2.5. Synthesis of Regioregular Poly(3-Decylthiophene)

The homopolymer, P3DT was made by Grignard metathesis reaction using EtMgBr as the initiator according to the method of McCullough *et al.* [27] with a slight modification. 2,5-dibromo-3-decylthiophene (1.65 g, 4.3 mmol) was dissolved in freshly distilled THF (25 mL) in a three-necked, 50 mL round bottom flask. The flask was equipped with a reflux condenser fitted with a calcium chloride drying tube as well as a nitrogen bubbler and placed in an ice bath. The initiator, ethyl magnesium bromide (0.6 mL, 4.5 mmol) was added to the reaction flask by means of a nitrogen purged hypodermic syringe and taken out of the ice bath. The flask was refluxed for about an hour after which the catalyst, Ni(dppp)Cl_2 (23 mg, 4.3 × 10^{-5} mol) was added in one portion to the reaction vessel and reflux continued for two more hours. The mixture was precipitated into methanol and isolated by centrifugation. Sequential Soxhlet extractions were then carried out in methanol, hexanes and finally chloroform. The polymer was obtained from the chloroform fraction after evaporation to dryness, in vacuo. Yield > 90%. ^{1}H NMR ($CDCl_3$, TMS) δ (ppm): 0.91–0.94 (t 3H), 1.27–1.75 (m 16 H), 2.66 (t 2H), 6.90.

2.6. Preparation of P3DT with Proton End Group

The homopolymer, P3DT (0.6 g) was dissolved in THF (50 mL) in a round bottom flask. Ethyl magnesium bromide (0.4 g, 0.02 mmol) was added to the flask and the mixture was refluxed for 2 h after which it was cooled to room temperature. In order to neutralize the reaction, HCl (2 M 5 mL) was added to the flask and this was followed by precipitation into methanol. The product was isolated by centrifugation and washed by Soxhlet extraction in methanol. The proton end-capped polymer was dried in the oven. Yield > 90%. ^{1}H NMR (CDCl3, TMS) δ (ppm): 0.80 (t 3H), 1.25 (t 3H), 1.26–1.60 (m 16H), 2.60 (m 2H), 2.75 (m 2H), 6.80 (s 1H).

2.7. Preparation of P3DT with Carbonyl End Group

The end group modification of the polymer was continued in order to convert it to a carbonyl end-capped polymer. P3DT with H-end group (0.4 g) was dissolved in toluene (100 mL) in a round bottom flask equipped with a condenser, drying tube, nitrogen inlet and bubbler. *N*-methylformamide (2 mL, 16 mmol) and phosphorous oxychloride (1.3 mL, 14 mmol) were added and the reaction was stirred at 75 °C for 24 h. The flask was cooled to room temperature and the mixture was stirred with saturated sodium acetate solution for two hours after which it was precipitated in methanol. The carbonyl end functionalized polymer was washed in methanol by Soxhlet extraction and dried in the oven. Yield ~ 80%. ^{1}H NMR ($CDCl_3$, TMS) δ (ppm): 0.80 (t 3H), 1.26–1.60 (m 16H), 2.60 (m, 2H), 2.86 (m 2H), 2.75 (m 2H), 6.80 (s 1H), 8.60 (CHO).

2.8. Preparation of P3DT with Hydroxyl End Group

The carbonyl end functionalized polymer was converted to hydroxyl end-capped polymer as follows: P3DT with CHO end group (0.35 g) was dissolved in THF (50 mL) in a round bottom flask equipped with a nitrogen inlet and bubbler. 1M $LiAlH_4$ (1 mL) was added via a purged hypodermic syringe and reaction stirred at room temperature for about an hour. It was then terminated by precipitation into methanol and compound was isolated by centrifugation. It was subsequently washed by Soxhlet extraction in methanol and oven dried afterwards to yield the hydroxyl end-capped P3DT. Yield > 70%. ^{1}H NMR (CDCl3, TMS) δ (ppm): 0.80 (t 3H), 1.26–1.60 (m 16H), 2.60 (m, 2H), 2.86 (m 2H), 1.82 (m 2H), 3.50 (m 2H), 6.75 (s 1H).

2.9. Preparation of Bromoester End Capped P3DT

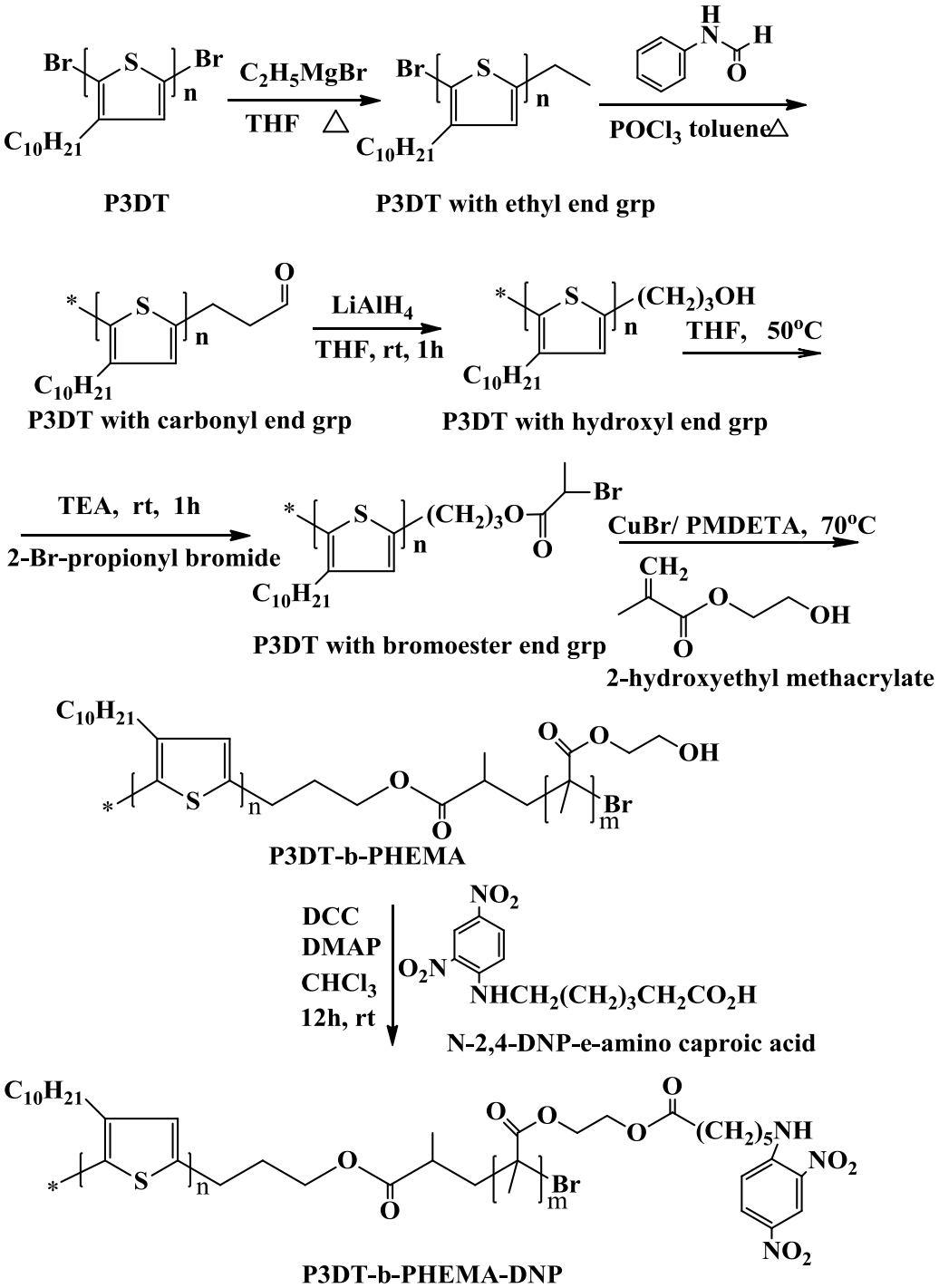
The bromoester end group P3DT was prepared by dissolving hydroxyl end-capped P3DT (0.3 g) in THF (50 mL) in a round bottom flask and heated to 50 °C. Triethylamine, TEA (3 mL, 20 mmol) was added by means of a hypodermic syringe and 2-bromopropionyl bromide (2.5 mL, 20 mmol) was added to the reaction in a drop-wise manner. The reaction was left to stir at room temperature for 12 h and terminated afterwards, by precipitation into methanol. The compound obtained was washed with cold methanol and oven dried. Yield ~ 90%. ^{1}H NMR ($CDCl_3$, TMS) δ (ppm): 0.80 (t 3H), 1.26–1.60 (m 16H), 1.90 (d 3H), 1.97 (m 2H), 2.60 (m, 2H), 2.86 (m 2H), 4.1 (m 1H), 4.40 (m 2H), 6.95 (s 1H).

2.10. ATRP of 2-Hydroxyethyl Methacrylate with P3DT Macroinitiator

The atom transfer radical polymerization of 2-hydroxyethyl methacrylate (HEMA), was carried out using the bromoester terminated P3DT as macroinitiator in the ratio of 300:2:1:1 (HEMA:P3DT-MI: CuBr:PMDETA) (Scheme 2). 50.3 mg of P3DT functionalized with the bromoester end groups macroinitiator was placed into a 25 mL flask, 156 mg of purified CuBr and 188.6 mg of PMDETA were added and the flask was sealed, filled and back-filled with nitrogen three times. DMF, 10 mL was added through a nitrogen purged hypodermic syringe and the resulting mixture was stirred. 2-Hydroxyethyl methacrylate (1.61 g) was added to the flask via a purged hypodermic syringe as well and the mixture was transferred to an oil bath maintained at 70 °C with constant stirring. The reaction

was then allowed to run for 20 h. Termination was done by precipitation into methanol and product was washed with hexanes by Soxhlet extraction. Yield ~ 80%.

Scheme 2. Synthetic route to DNP-functionalized poly(2-hroxyethyl methacrylate)-b-poly(3-decylthiophene).



2.11. Functionalization of P3DT-b-PHEMA with DNP Groups

The functionalization of the block copolymers with N-2,4-DNP-ε-amino caproic acid was done as reported by Sannigrahi *et al.* [10] with some modification. The copolymers (25 mg) were placed in a three-necked flask. To this was added *N*-2,4-DNP-ε-amino caproic acid (0.7 mg, 2.5 mmol), DCC

(7.5 mg, 3.75 mmol) and DMAP (0.1 mg). The flask was placed under dry nitrogen for 2 h after which 5 mL dry chloroform was introduced into the flask using a nitrogen purged hypodermic syringe. The reaction was then left to run for 12 h with constant stirring. Thereafter, the reaction mixture was filtered and purified by sequential precipitation from hexanes and methanol and isolated by centrifugation. Yield > 85%.

2.12. Polymer Processing

The DNP functionalized block copolymers were processed into thin films by drop-casting from chloroform solution. 1 mg of DNP-PHEMA-b-P3DT was dissolved in 1 mL chloroform. The mixture was drop-cast onto a piece of silicon wafer and dried under ambient conditions.

2.13. Biofunctional Property Studies

2.13.1. Block Copolymers from 3-Decylthiophene and 2-Hydroxyethyl Methacrylate Functionalized with DNP

The polymer-film interaction with IgE was investigated by incubating the films in a solution of fluorescently tagged anti-DNP IgE in 1% phosphate buffered saline, PBS, for one hour. It was then rinsed copiously with PBS buffer solution three times and then imaged using a Zeiss Axiovert 200M Fluorescence/Live Cell Imaging Microscope.

2.13.2. α, ω-bi-Biotin (Poly (Ethylene Oxide)-b-Poly(Styrene)-b-Poly (Ethylene Oxide)) Block Copolymers

The unfunctionalized polymer and the biotin functionalized polymer were dissolved in THF (1% solution) and coated on microscopic glass. The unbound sites were blocked by incubating the slides for 20 min at room temperature in 1% BSA. Then the slides were washed in buffer three times, 5 min each to remove the surface unmasked BSA. Finally, the slides were incubated in AMCA Avidin (Excitation: 345–355 nm; Emission 448–454 nm) from Vector Laboratories Inc. (Burlingame, CA, USA), diluted with buffer (1:200) for 30 min at room temperature. Then the slides were washed in buffer three times, 5 min each to remove the surface unmasked AMCA Avidin and mounted using Vectasheild anti-fade fluorescent mounting media from Vector Laboratories Inc. and viewed and imaged using Carl Zeiss Axio Imager Z.1 microscope using AxioVision 4.8.2 (2010) software. Bicarbonate buffered saline, pH 8.2 was used everywhere as the working buffer solution.

2.14. Characterization/Instrumentation Techniques

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE III 500 MHz and Bruker AVANCE 400 MHz NMR spectrometers in deuterated chloroform, $CDCl_3$ and dimethyl sulfoxide, DMSO-d_6 respectively. The $CDCl_3$ contained 0.05% v/v tetra-methylsilane, TMS, as internal standard while DMSO-d_6 contained 0.03% v/v TMS. All chemical shifts were referenced to TMS.

UV-Vis absorption spectra were recorded with a Varian Cary 500 Scan UV-Vis-NIR Spectrophotometer. Stock solutions of polymer samples (1 mg/5 mL) were made from spectrophotometric grade chloroform, purchased from Aldrich chemicals.

FTIR spectra were recorded on a Perkin Elmer Spectrum 65 FT-IR spectrometer using sample pellets obtained by pressing polymer samples with KBr.

Thermal analysis was done with TA Instrument Q2000 Differential Scanning Calorimeter at heating rate of 10 ℃/ min.

Light Scattering analysis was carried out with the DynaPro Nanostar from Wyatt Technology at 25 ℃ and samples were prepared in HPLC grade chloroform. Prior to use, the samples were filtered with 0.10 μm Whatman syringe filters. Analysis was done in a quartz cell.

Agilent Technologies 8500 Field Effect-Scanning Electron Microscope (FE-SEM) was also used for morphology characterization of the thin films.

The electronic characteristics of the polymers were investigated via cyclic voltammetry with the PARSTAT 2263 Advanced Electrochemical System from Princeton Applied Research in 1 mg/mL of sample in acetonitrile under a nitrogen sealed atmosphere at ambient temperatures. Reference electrode was Ag/AgCl, NaCl saturated. Scanning rate of 50mV/s was used.

X-ray photoelectron spectroscopy (XPS) spectra were obtained using a Thermo K-Alpha XPS.

Size exclusion chromatography (SEC) was performed using a Perkin Elmer Binary LC pump 250, a 2792 injector and Perkin Elmer LC-30 RI detector. Three columns, HR3, HR 4E, HR 5E, were used in conjunction with 2 μm precolumn filter. The columns were housed in an oven maintained at 30 ℃. Tetrahydrofuran was used as the eluent at a flow rate of 1 mL/min. Molecular weights were calculated relative to polystyrene (Aldrich) molecular weight standards

3. Results and Discussion

3.1. Characterization of α, ω-bi-Biotin(Poly(Ethylene Oxide)-b-Poly(Styrene)-b-Poly(Ethylene Oxide))

The functional polymers were obtained in yields of 90% or higher as a white powder after precipitation into hexane. Table 1 lists the composition and molecular weights of polymers prepared by Scheme 1. The ^{1}H NMR of the tri-block copolymers are shown in Figure 1A. In the NMR spectrum the peaks at 1.3 and 1.7 ppm are attributed to the CH_2 and CH protons of polystyrene. The peaks at 7.0 and 6.5 ppm are credited to the aromatic protons in styrene. The sharp peak at 3.7 ppm is that of PEO.

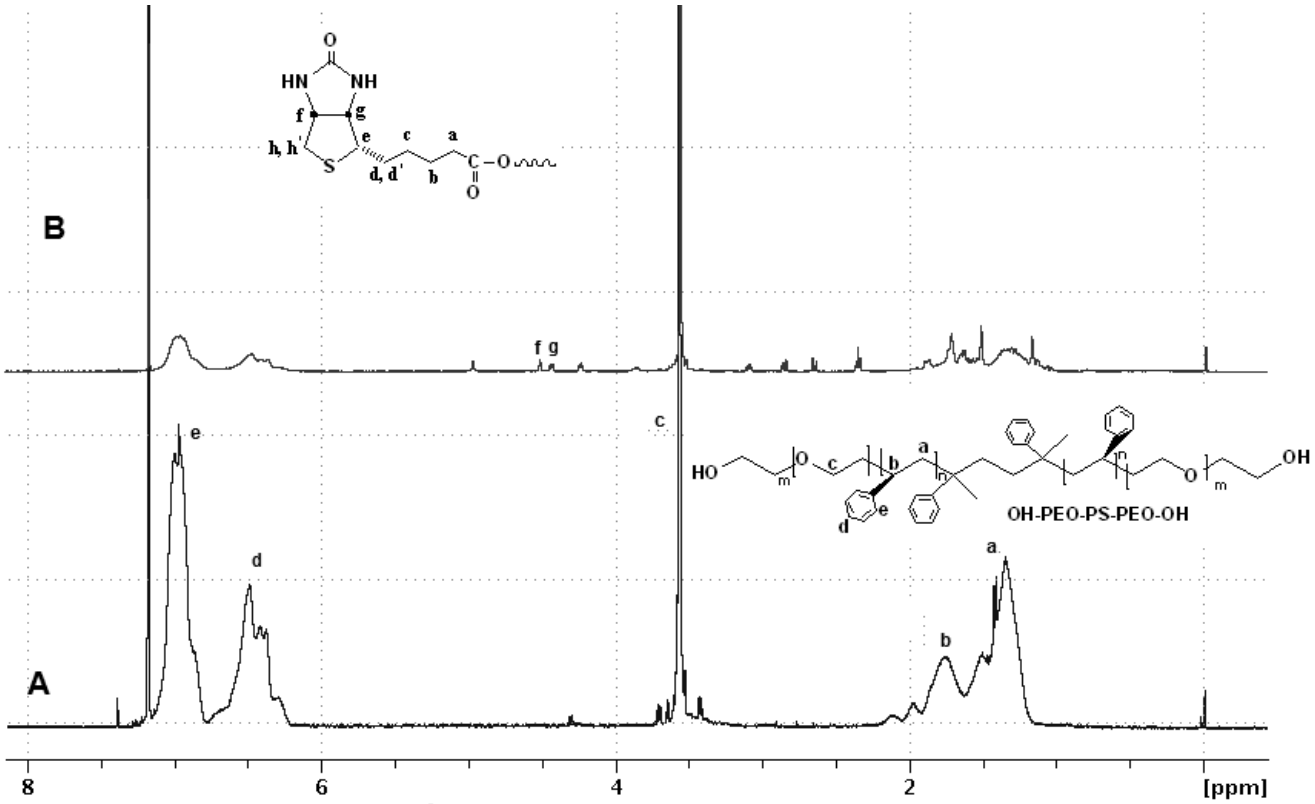
Table 1. Composition and molecular weights of α, ω-bi-hydroxyl(poly(ethylene oxide)-b-poly(styrene)-b-poly(ethylene oxide)).

Sample	M_1/M_2 (^{1}H NMR)	M_n (GPC)	M_w (GPC)	PDI	M_n (^{1}HNMR)
Sample 1	21/79	15k	20k	1.34	15k
Sample 2	21/79	18k	22k	1.24	13k
Sample 3	31/69	13k	25k	1.91	13k
Sample 4	32/68	-	-	-	33k

FT-IR spectroscopy was used to determine the end functional groups before and after functionalization. Hydroxyl functional groups stretching was observed at 3500 cm^{-1} for all of the polymers listed in Table 1 after termination with methanol. After coupling with biotin the hydroxyl group disappears, this is indicative of quantitative functionalization. An amine stretching around 3400 cm^{-1} along with an unread peak at 1600 cm^{-1} was observed after complete functionalization of the polymers. ^{1}H NMR

was also used to determine the end functionalization of biotin at each chain end. Shown in Figure 1B is the spectrum of Biotin-PEO-PS-PEO-Biotin. At 4.3 (f) and 4.6 (g) ppm are attributed to the protons in biotin respectively.

Figure 1. 500 MHz ^{1}H NMR ran using $CDCl_3$ and tetramethylsilane as an internal standard A. HO-PEO-PS-PEO-OH B. Biotin-PEO-PS-PEO-Biotin.



The molecular weights of all polymers were determined using gel permeation chromatography (GPC) relative to polystyrene standards. The molecular weights were determined using THF as the eluent as the polymers are soluble in this solvent. For lower molecular weight polymers, distributions were slightly broad (1.3–1.9). In contrast, the molecular weight distribution for higher molecular weight polymers was narrow (1.24) displaying a sharp peak.

To determine the thermal properties, differential scanning calorimetry (DSC) studies were carried out over the temperature range of 25 to 200 ℃. Figure 2 shows the DSC thermograms for the precursor polymer OH-PEO-P2MS-PEO-OH and after functionalization with Biotin (Biotin-PEO-P2MS-PEO-Biotin). All the polymers exhibited a glass transition temperature around about ~95 ℃ which is attributed to the polystyrene block. However, there was melting temperature present at ~50 ℃ for the PEO block, which indicates microsphase of the blocks are separated. The functionalized polymers displayed a sharp transition at 170 ℃, signifying the decomposition of biotin as the protein's melting temperature is around 233 ℃.

The elemental composition of the OH-PEO-P2MS-PEO-OH fibers were analyzed using x-ray photoelectron spectroscopy (XPS) shown in Table 2. The C1s and O1s signals appeared at 294 and 533 eV respectively. The carbon signal is accredited to the carbon backbone of PS and PEO. The Oxygenated species is due to the hydroxylated end groups of the tri-blocks. A Sn3d signal at 487 eV

indicates that there's a slight contamination of tin on the fiber's surface. This contamination was less than 0.1 atom.% which is very insignificant (this may arise from tin particles floating in the air). The presence of the AuNPs is evident in the survey scan in Figure 3 around 100 eV.

Figure 2. DSC thermograms of A. OH-PEO-P2MS-PEO-OH B. Biotin-PEO-P2MS-PEO-Biotin.

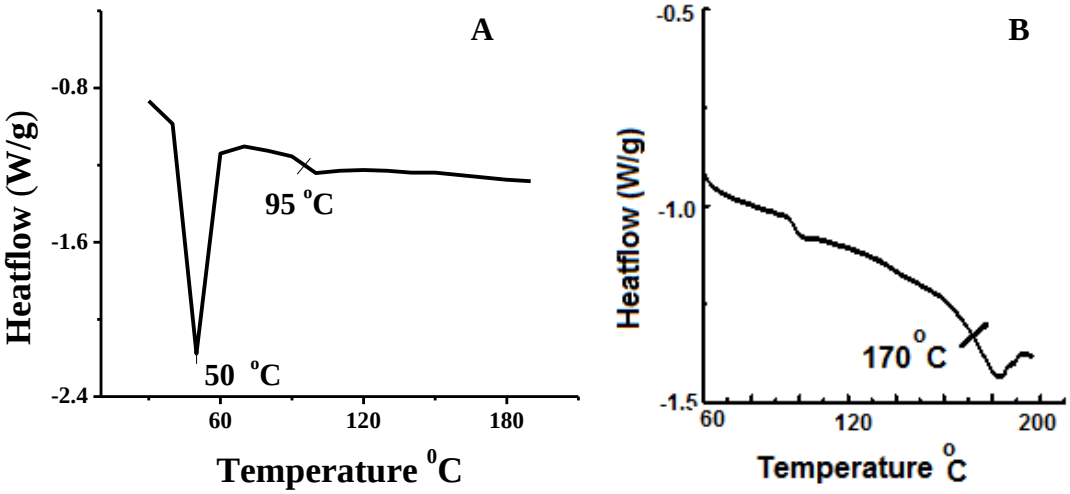
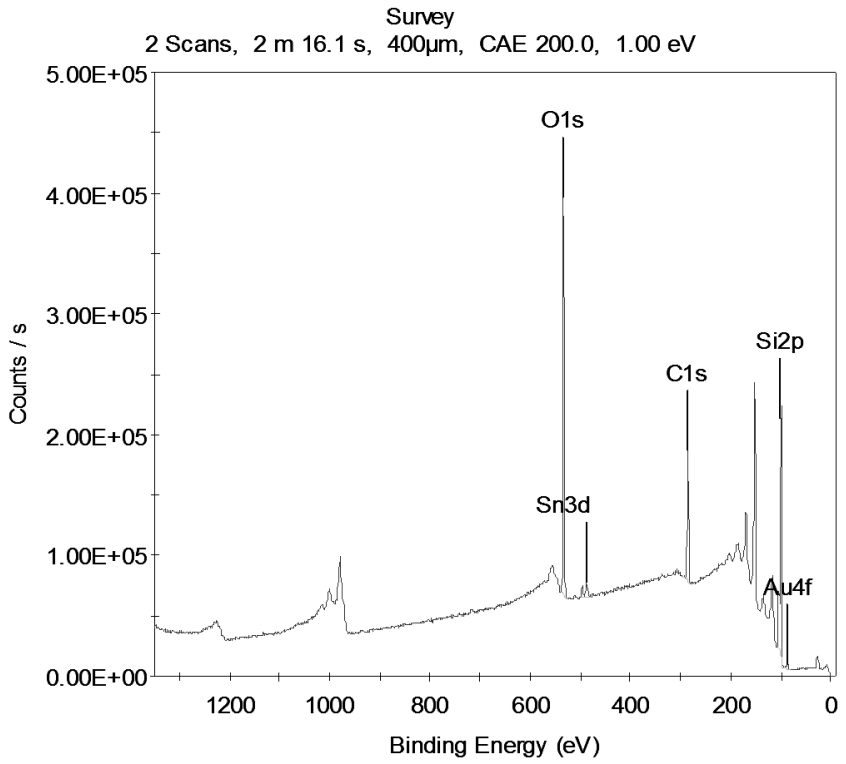


Table 2. XPS measurements of fibers made using sample 3 and thiolated AuNPs.

Samples	Atom	Peak (eV)	FWHM (eV)	Atom %
13k AuNPs fibers	O1s	533.16	2.83	27.25
	Si2p	100.22	2.61	43.72
	C1s	294.08	3.28	25.01

Figure 3. Survey scans of fibers comprised of Sample 3, Table 1 with thiolated AuNPs.



3.2. Characterization of Block Copolymers from 3-Decylthiophene and 2-Hydroxyethyl Methacrylate Functionalized with DNP

Copolymerization of regioregular poly(3-alkylthiophenes) with vinyl polymers is a way of improving the processability of the vinyl polymers. A method of synthesis, especially with methacrylate polymers, which are not easily soluble, is to copolymerize them with readily soluble polythiophene with long alkyl side chains. This can be achieved by first polymerizing the thiophene block by Grignard metathesis (GRIM) reaction and modifying the end groups to yield a species (haloester) that will readily react with the vinyl monomer by acting as a macroinitiator to form copolymers.

Regioregular poly(3-decylthiophene) was prepared via GRIM reaction and this was followed by magnesium-halogen exchange reaction with a Grignard reagent to obtain a proton capped polymer. Formation of this H-capped polymer was confirmed by ^{1}H NMR and the polymer subsequently went through a series of end group modifications until it was finally converted to a bromoester end-capped macroinitiator. ATRP of 2-HEMA was carried out with the P3DT-macroinitiator, Cu catalyst and PMDETA ligand to yield the block copolymers. Functionalization of the block copolymers with *N*-2,4-DNP-ε-amino caproic acid resulted in the DNP functionalized block copolymers (Scheme 2). The degree of functionalization estimated from ^{1}H NMR data indicated that the copolymers were 50% functionalized.

Figure 4. 500 MHz ^{1}H NMR spectrum of PHEMA-b-P3DT and DNP functionalized P3DT-b-PHEMA.

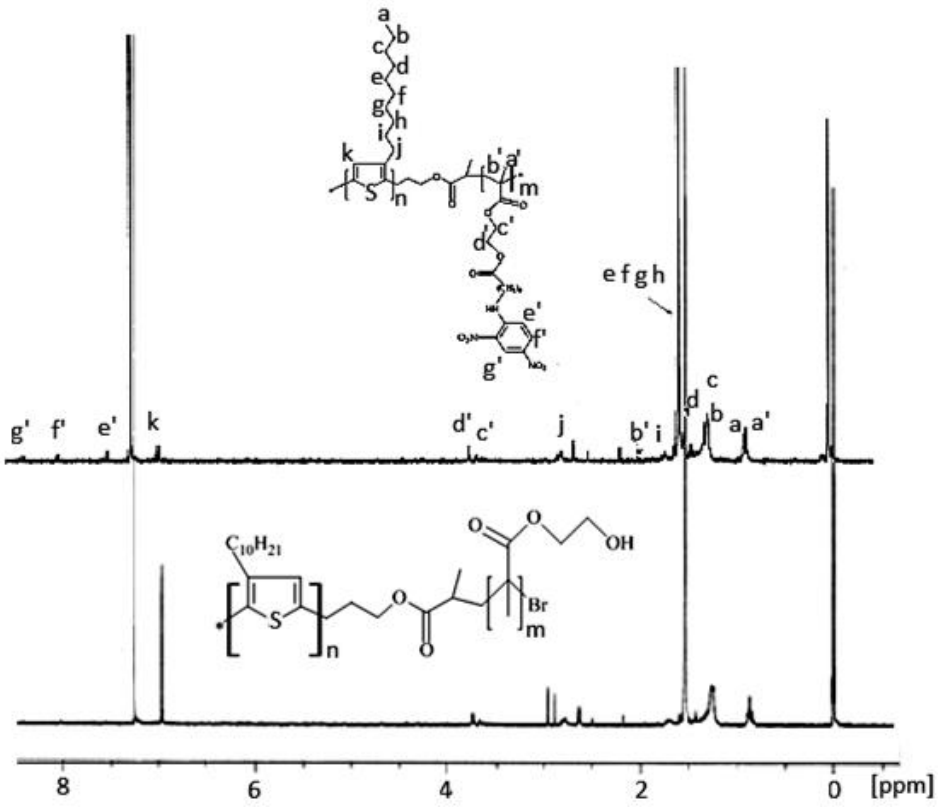
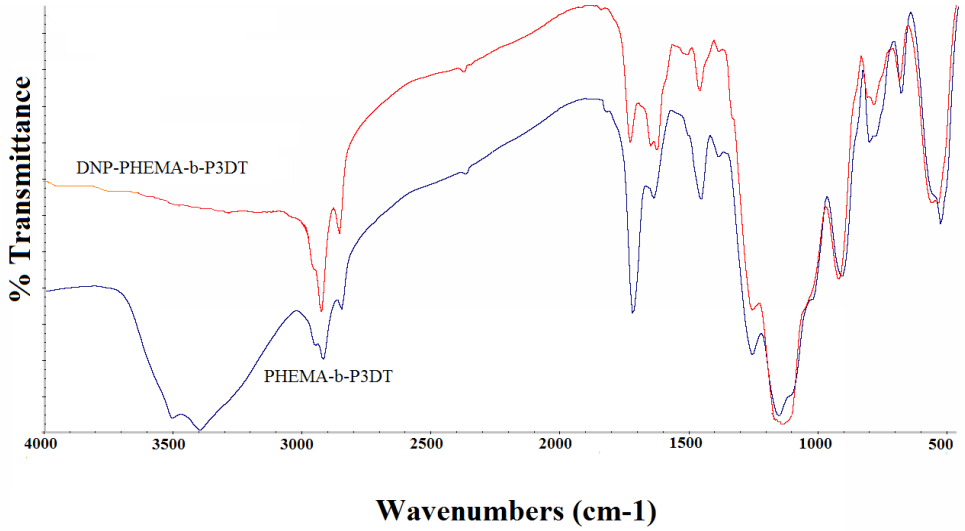


Figure 4 is the ^{1}H NMR spectrum of the block copolymers, PHEMA-b-P3DT and the ^{1}H NMR spectrum of the DNP- functionalized poly(2-hydroxyl ethyl methacrylate)-b-poly(3-decylthiophene). Both

^{1}H NMR spectra show their peaks at the expected chemical shifts, and this indicates that the target products were synthesized.

The infrared spectra for the copolymers (blue) and the DNP-functionalized co-polymers (red) are shown in Figure 5. The aliphatic C–H stretching for the P3DT block appears at ~1500 cm^{-1}. The methyl rock, also for P3DT appears at ~600 cm^{-1} while the aromatic C–H out of plane is found at ~800 cm^{-1}. The C–S bending frequency can be found at ~1700 cm^{-1}. The moderately broad but very intense peak with shoulders between ~1000 cm^{-1}–1300 cm^{-1} belongs to the –C–O–C—in PHEMA [28].

Figure 5. FTIR spectra of DNP functionalized PHEMA-b-P3DT and PHEMA-b-P3DT.



The symmetric stretching for the –NO_2 is found at ~1320 cm^{-1} for DNP. The aromatic C–H stretching frequencies for P3DT can be seen at ~2800, 2900 and 2920 cm^{-1}. The sharp peak seen at ~1710 cm^{-1} belongs to carbonyl (C=O) absorption band on PHEMA. The broad band in the triblock co-polymers from ~3000 to 3700 cm^{-1} corresponds to the OH vibrations of the side groups in the PHEMA block [29]. After the block co-polymers were functionalized with DNP-ε-amino-*n*-caproic acid, the OH peak for PHEMA did not show up, thus, confirming its replacement with the DNP functional group. The characteristic peaks of the copolymers are indicated on the spectra.

The Raman spectrum for the DNP- functionalized copolymer is found in Figure 6. The sharp and intense peak at ~500 cm^{-1} is attributed to the C–C aliphatic chain vibrations on the P3DT blocks. The medium peak found at ~950 cm^{-1} is the C–O–C symmetric vibration while that seen at ~1150 cm^{-1} is assigned to the asymmetric vibration of the same group. Delta 1380 cm^{-1} is attributed to the CH_3 and delta at 1450 to 1500 cm^{-1} are for CH_2 and asymmetric CH_3. The C–NO_2 asymmetric peak is between 1340 to 1380 cm^{-1} [30].

Ultraviolet-visible spectroscopy deals with the electronic transitions of molecules when they absorb or reflect in the ultraviolet-visible spectral region of the electromagnetic spectrum. UV-Vis spectroscopy is based on the Beer Lambert's law which states that the absorbance of a solution is dependent on the concentration of the absorbing species in the solution as well as the path length. Samples of the block copolymers and DNP- functionalized copolymers were dissolved in chloroform and analyzed by UV-Vis spectroscopy. Figure 7 shows the UV-Vis spectra of PHEMA-b-P3DT and DNP-PHEMA-b-P3DT. The maximum wavelength of absorption, λ_{max}, is observed at ~445 nm. The λ_{max} experienced a

slight shift to 450 nm from 445 nm (for the non-functionalized polymers) which corresponds to a bathochromic shift. The shoulder seen just before the maximum absorption is attributed to the DNP functional group present in the block copolymers. This is a confirmation that the polymers are indeed functionalized with the intended DNP groups.

Figure 6. Raman spectrum of the DNP-functionalized PHEMA-b-P3DT.

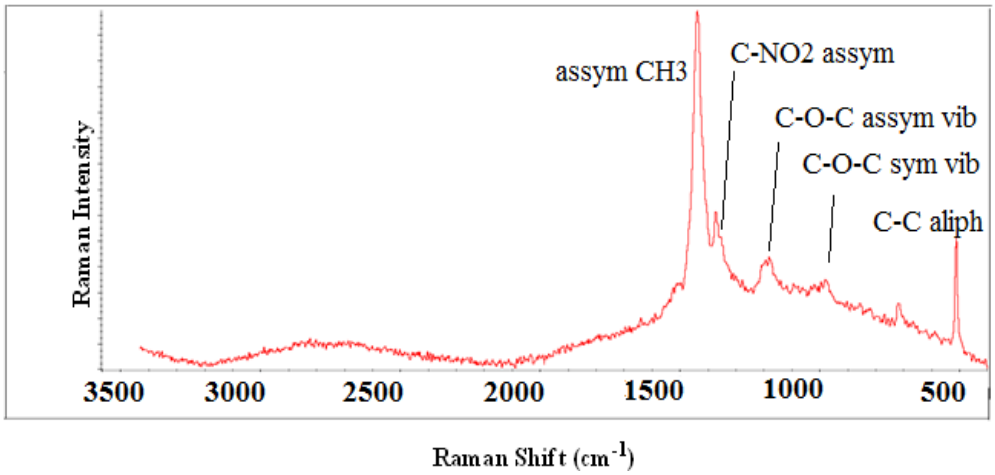
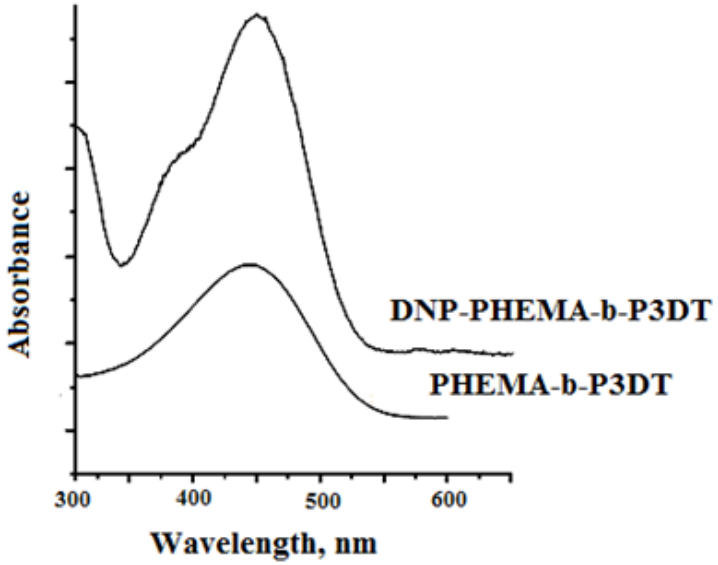
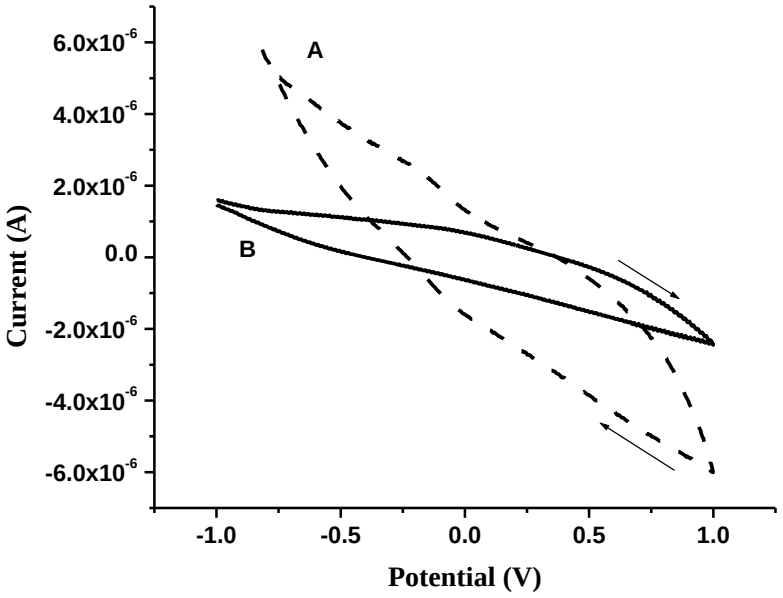


Figure 7. UV-Vis spectrum of PHEMA-b-P3DT and DNP-PHEMA-b-P3DT.



Cyclic voltammetry, C–V, is the electrochemical measurement of a species in solution. C–V occurs by cycling the potential of a working electrode and measuring the corresponding current. It is pertinent that for cyclic voltammetry to be of use, the species being analyzed has to be redox active within the experimental potential range. Figure 8A is the cyclic voltammogram of PHEMA-b-P3DT. In the spectrum, the copolymer is seen to cycle up to 1.0 V. It shows a very broad oxidation peak seen at ~0.25 V while the reduction peak, not so broad is seen at ~−0.20 V. This redox couple can be ascribed to poly(3-decylthiophene) species [31,32].

Figure 8. Cyclic voltammogram of A. PHEMA-b-P3DT B. DNP-PHEMA-b-P3DT.



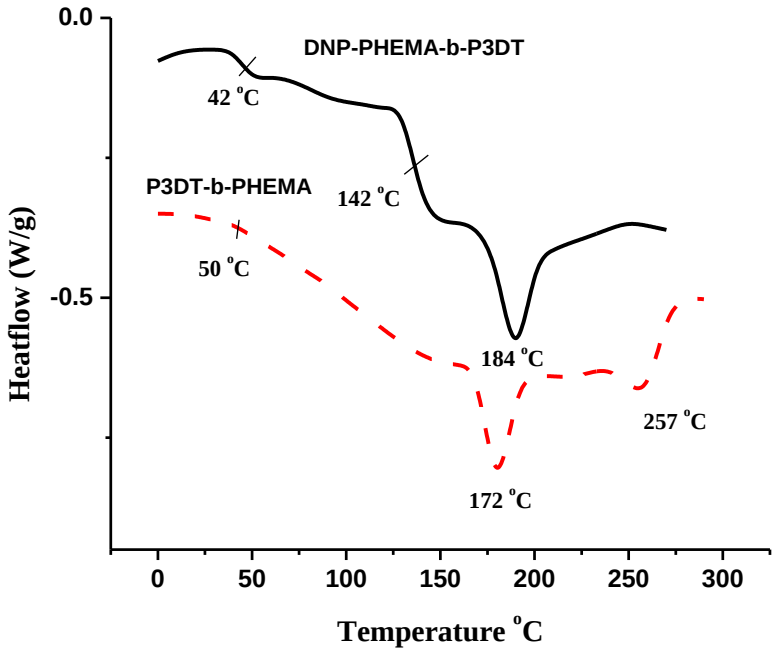
In the DNP functionalized copolymers (Figure 8B), cycling is seen up to 1.0 V, just as observed previously for the copolymers. The spectrum shows a very broad peak assigned to the oxidation of the copolymers at ~0.75 V. Its reduction peak, however, shows up at a potential of ~0.15 V which is of course higher than the reduction peaks of the homopolymer and non-functionalized copolymers. This big difference, is caused by the presence of DNP groups in the copolymers. This type of shift in peak potential, has been reported in literature in a polythiophene bearing a nitrobenzene group [33]. Both polymers were found to be redox active.

Differential scanning calorimetry, DSC, is a thermal analytical technique which measures the amount of heat required to raise the temperature of a sample relative to a reference, as a function of temperature. Information about physical transformations (or phase transitions) that transpire in a material as it is heated or cooled to a certain temperature is given in a DSC experimental scan. Parameters such as glass transitions and heat capacity can be measured by this thermal technique and it has gained ground in the industry as a quality control tool to determine material purity and investigation of curing in polymers.

Figure 9 is the DSC scan of P3DT-b-PHEMA copolymers and DNP-PHEMA-b-P3DT. Two endotherms are observed. The endotherm, a sharp peak at ~172 ℃ is assigned to the melting of the PHEMA block, while the small broad peak at ~257 ℃ is assigned to the thiophene block. This is consistent with the thiophene ring melting temperature seen in the thermogram of the homopolymer. No crystallization was seen in the copolymers. The glass transition was not very obvious. It appears at about 50 ℃ slowly leveling out till the onset of melting of the PHEMA block. The two endotherms observed, confirmed the formation of the diblock copolymers.

The DNP functionalized polymers show an endotherm at 184 ℃, which is just slightly higher than the T_m seen in the non-functionalized copolymers for the PHEMA block. This is likely due to the presence of DNP groups leading to a more cohesive bond between the two and so, the movement of PHEMA chains, is restricted. This would require more energy to make the PHEMA molecules move. However, there is a slight transition at about 42 ℃, which is attributed to the Tg of P3DT, while there is a distinct endotherm at ~142 ℃ which is ascribed to the T_g for the DNP-functionalized PHEMA.

Figure 9. DSC thermograms of P3DT-b-PHEMA and DNP-PHEMA-b-P3DT.



An efficient method of determining the size distribution profile of particles of a polymer in solution is by dynamic light scattering, DLS. It is also used to determine molar mass, among other functions. It shows a bi-modal distribution of the particle radii with the small fraction having average radius of ~2.5 nm, while the majority of the copolymer particles have an average hydrodynamic radius of ~18.6 nm. Hence, the block copolymers of P3DT-b-PHEMA, have an average size of 36 nm in diameter and estimated molecular weight of 130 kDa.

The morphology of the copolymer film as observed by SEM shows a rough surface with pores spread all over. This is expected because of the nature of the PHEMA block as it is jelly like. The appearance of the film is consistent with the report of Senthilkumar *et al.* [34].

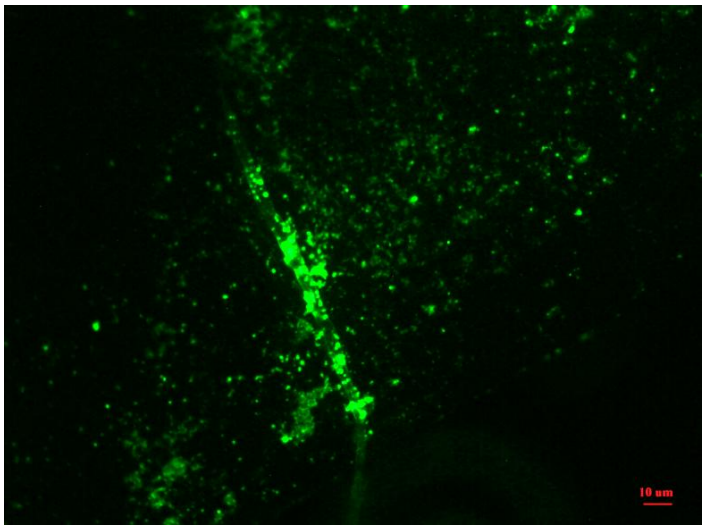
3.3. Functional Polymer-Protein Interactions

The two sets of functional block copolymers have been carefully characterized and the integrity of the structures ascertained.

3.3.1. Block Copolymers from 3-Decylthiophene and 2-Hydroxyethyl Methacrylate Functionalized with DNP

The IgE antibody binds onto specific antigens through the Fc-receptors present. These receptors recognize certain moieties to which they are active and when present, they become attracted and therefore bind to them. In this study, anti-DNP IgE was used and so, the DNPs on thin film were recognized and binding occurred. Figure 10 shows the fluorescence microscope image of IgE bound film of DNP functionalized PHEMA-b-P3DT co-polymers. Here, specific binding of IgE to the DNP groups is inferred as not all the surface of the film show fluorescence.

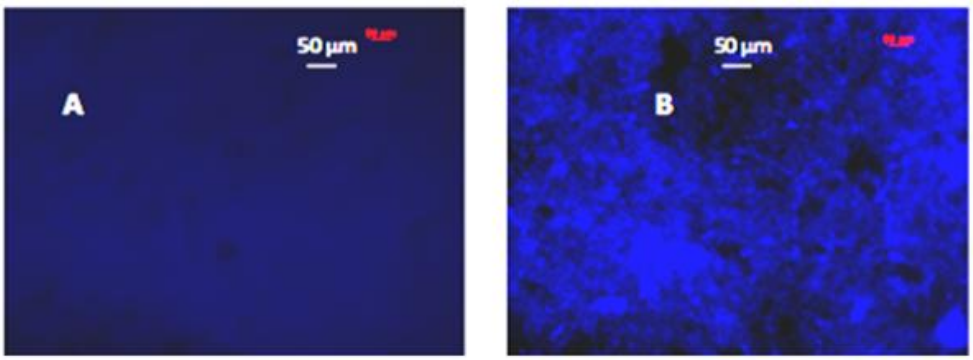
Figure 10. Fluorescence micrograph of a film of DNP-PHEMA-b-P3DT after incubation in FITC-IgE.



3.3.2. Binding of Avidin with Surfaces Prepared from Block Copolymers with Biotin and without Biotin

Fluorescence tagged avidin antibody binding studies were conducted on unfunctionalized polystyrene-b-poly(ethylene oxide) block copolymer as control A and biotin functionalized block copolymer as sample B. These block copolymers are composed of hydrophobic polystyrene segment and hydrophilic poly(ethylene oxide) segment. In antibody binding, hydrophobic interactions between hydrophobic segment of block copolymer and antibody play an essential role. These forces promote non-specific binding. Bovine serum albumin (BSA) treatment is a common procedure used to reduce non-specific hydrophobic bindings. Both the sample slides were treated with 1% BSA to eliminate non-specific binding. Finally, the slides were incubated with AMCA Avidin with emission frequency of 448–454 nm. Following incubation with the AMCA Avidin, antibody binding could be visualized and imaged using a fluorescent microscope. No significant fluorescence was observed with control A.

Figure 11. Fluorescence micrographs of a film of α, ω-bi-biotin (poly(ethylene oxide)-b-poly(styrene)-b-poly(ethylene oxide)) block copolymers after incubation with Avidin. (**A**) control; (**B**) Sample.



It confirms that all non-specific binding sites due to hydrophobic interactions were successfully blocked with treatment of 1% BSA. Sample B has been characterized with strong blue fluorescent

(Figure 11). This signifies that Avidin is specifically bound to antigen Biotin [35]. These observations lead to two important conclusions that the block copolymers were successfully functionalized with Biotin and biotin is exposed on the surface of the polymer film so that makes it available for binding.

4. Conclusions

Two series of biofunctional block copolymers have been synthesized and characterized. The electroactive block copolymer composed of poly(3-decylthiophene), P3DT and poly(2-hydroxy ethyl methacrylate), PHEMA and functionalized with DNP, showed the thermal behavior indicative of a microphase separated block copolymer matrix. The polymers also showed interesting electrochemical features, one of which is the redox potential of the DNP functionalized polymers being shifted away from those of the homopolymer and copolymers. Incubation of the block copolymers films in IgE for an hour followed by washing with phosphate buffered saline solution and observation under the fluorescence microscope indicated the binding specificity of IgE with the functional copolymers through its FcεRI receptors. Additionally, films of α, ω-bi-biotin (poly(ethylene oxide)-b-poly (styrene)-b-poly(ethylene oxide)) also showed specific binding with avidin. The results suggest that functionalized block copolymers are effective in fabricating films and structures with decorated functional groups capable of specific interaction with biomarkers. These electroactive polymers have great potential as biosensors and diagnostic agents.

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Author Contributions

O.O. and L.L. performed the experiments and contributed to writing, B.S. helped with experiment and writing, P.N. helped with studies, M.W. helped with voltammetry and writing. I.K. designed, supervised and contributed to writing.

Conflicts of Interest

The authors declare no conflict of interest.

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