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Synthesis and Morphological Characterization of Block Copolymers for Improved Biomaterials

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Abstract

Biocompatible polymers are known to act as scaffolds for the regeneration and growth of bone. Block copolymers are of interest as scaffold materials because a number of the blocks are biocompatible, and their nanostructure is easily tunable with synthetic techniques. In this paper, we report the synthesis of a novel class of biomaterials from block copolymers containing a hydrophobic block of methyl methacrylate and a hydrophilic block of either acrylic acid, dimethyl acrylamide, or 2-hydroxyethyl methacrylate. The block copolymers were synthesized using a combination of reversible addition-fragmentation chain transfer (RAFT) polymerization and click chemistry. Since the surface morphology is critical for successful cell growth, atomic force microscopy (AFM) studies were conducted for selected block copolymers. The topography, phase angle and friction maps were obtained in dry and physiological buffer environments to study the morphology. Results of AFM imaging identified the presence of polymer domains corresponding to the copolymer components. The distribution of nanoscale features in these block copolymers are comparable to those found on other surfaces that exhibit favorable cell adhesion and growth. In physiological buffer medium, the hydrophilic component of the block copolymer (acrylic acid or hydroxyethyl methacrylate) appear to be present in greater amounts on the surface as a consequence of water absorption and swelling.

Keywords

block copolymers; RAFT; click coupling; atomic force microscopy

1. Introduction

In dentistry, bone loss occurs in a number of ways. For instance, diseases such as aggressive periodontitis could lead to the degradation and eventual loss of the alveolar bone under the teeth [1]. Injuries, such as those resulting from accidents, can also lead to bone loss which would require regeneration treatment using scaffolds, as shown in the cartoon in Figure 1 (adapted from [2]). One of the goals of bone tissue engineering (TE) is to use scaffolds as a template to guide regeneration. TE scaffolding can potentially treat critical sized defects in

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bone or hard tissue. Scaffolds serve as templates for bone regeneration and their surfaces support the attachment and growth of cells that form new bone [3].

Many factors are known to influence the cell and tissue response to scaffolds, bulk surface chemistry [4], the presence of biological ligands [5] and microscale morphology [6–7] to name a few. The nanoscale topography of tissue engineering scaffolds is known to have a significant influence on the cellular and host response. A large number of studies have explored the cellular and host response to nanoscale features such as porosity and roughness or topology [8–12]. While the exact mechanism is not understood, it is clear that the cell surface receptors respond to nanoscale features.

Many synthetic motifs have been used to generate nanoscale topology; however, block copolymers remain a relatively unexplored system in biomaterials. Block copolymers are known to self-assemble into regular nanopatterns [13] and can potentially be utilized to regulate cell behavior. Block copolymers provide a bottom-up approach toward designing nanoscale biomaterials. Block copolymer morphology is a function of the composition and molecular weight of the individual blocks, as well as the spatial relationship of the blocks, for instance A–B block copolymers will have a different morphology than A-B-A block copolymers which may be different from comb or star architectures. By varying the molecular weight and spatial arrangement of the blocks, various polymer domain morphologies are observed, such as spheres, cylinders, lamellae and bicontinuous conformations [14]. Diverse morphologies can therefore be generated from a few simple building blocks. We will use methyl methacrylate (MMA), 2-hydroxyethyl methacrylate (HEMA), acrylic acid (AA) and dimethyl acrylamide (DMA) as the monomer building blocks. These building blocks were chosen because their homopolymers are used to make biocompatible materials and devices. For example, poly(methyl methacrylate) (PMMA) is a component of bone cements and poly(2-hydroxyethyl methacrylate) (PHEMA) is component of contact lenses. Poly(acrylic acid) (PAA) and poly(dimethyl acrylamide) (PDMA) are also used in biomaterials.

The diverse morphologies obtained from the block copolymers can potentially be used as models to understand cell-polymer interactions. Based on the molecular weight and the configuration of the blocks it is possible to create surfaces with the same chemical composition but different nanomorphologies. For example, a block copolymer with 50% A and 50% B could be synthesized in an A–B configuration or an A-B-A configuration. In the preceding example the chemical composition is the same but the nanomorphology is different. This would allow for a fundamental understanding of the role nanomorphology in cell-polymer interactions independent of chemical composition. Additionally, many biomaterials are composed of the above building blocks and the block copolymer motif can be used to design biomaterials with improved host interactions.

Many techniques for synthesizing block copolymers are known [15–17] however the preferred method for creating block copolymers from water-soluble free radically polymerized monomers is reversible addition-fragmentation chain transfer (RAFT). The mechanism of RAFT polymerization are the exchange of xanthates [18] and a wide variety of polymers with narrow polydispersities can be obtained. When combined with click chemistry, RAFT leads to the synthesis of controllable nanostructured biomaterials generated from phase separated block copolymers [19–20].

Click chemistry corresponds to an efficient and selective Cu (I) catalyzed variant of the Huisgen 1,3-dipolar cycloaddition of azides and alkynes reaction to form 1,4-disubstituted 1,2,3-triazole under moderate reaction conditions [21–27]. The utility of the click reaction has been demonstrated well in living radical polymerization systems. The functionalization

and subsequent modification of various substrates is provided by click functionalized polymers that have predictable architecture. For example, reports [28–32] have recently revealed such polymers which utilize RAFT and accompanying azide–alkyne coupling reactions for the synthesis of block copolymers. In effort to continue our research interests in the polymer chemistry [33–35] we report the synthesis of a series of well defined block copolymers PAA-b-PMMA (1/3, 1/1, 3/1 [mol/mol]), PDMA-b-PMMA (1/3, 1/1, 3/1), PHEMA-b-PMMA (1/3, 1/1, 3/1) from bioacceptable polymer blocks by combining RAFT and click chemistry.

In order to evaluate the potential of these block copolymers for scaffold applications, the morphology of the surface needs to be fully understood. In addition, there is a need to characterize the morphology of the block copolymer films while immersed in a buffer that simulates physiological conditions in order to predict the physical and chemical interactions of the individual polymer blocks with its environment for its intended application. In this paper, we address these issues by imaging surfaces of these biocompatible block copolymer films using atomic force microscopy (AFM). Aside from surface topography, AFM imaging allows the examination of the viscoelastic and friction properties of these films, which in turn, can be used to identify the different phases present. In addition, the morphology of these films under a physiological buffer is shown for the first time. These experiments will be valuable for future cell-material studies using these block copolymer systems.

2. Experimental

2.1 Materials and Synthesis Procedure

2.1.1 Materials—All solvents, monomers and other chemicals were purchased from Aldrich at the highest purity available unless otherwise stated. Methyl methacrylate (MMA), 2-hydroxyethyl methacrylate (HEMA), acrylic acid (AA) and dimethyl acrylamide (DMA) were passed through MMQ column to remove the inhibitors. 2, 2'-Azobis (isobutyronitrile) (AIBN, 97%) was purified by recrystallization from ethanol and dried at room temperature in a vacuum oven and stored in a freezer. Xylene was distilled and dried over molecular sieves (4 Å) before use.

2.1.2 Synthesis of Monomers and Polymers

2.1.2.1 Synthesis of RAFT CTAs: Raft reagent **1**, the structure of which is shown in Figure 2, was synthesized by following the published procedure [36] using decanethiol as the starting material. Azido-trithiocarbonate agent **2** and alkyne-trithiocarbonate agent **3** were synthesized by the coupling reaction of the raft reagent **1** with azido propanol and propargyl alcohol respectively using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) [37].

2.1.2.2 Homopolymerization of methylmethacrylate: To a solution of methylmethacrylate (1.52 g, 15.2×10^{-3} mol) in dry xylene (3 mL), was added AIBN (1.54 mg, 9.38×10^{-6} mol), and the azido-trithiocarbonate agent **1** (0.021 g, 4.69×10^{-5} mol) and nitrogen was bubbled for 15–30 min. The reaction mixture was warmed under nitrogen atmosphere to 80 °C for 24 h. The polymer was precipitated in hexane and the conversion was evaluated by FTIR and NMR.

2.1.2.3 Homopolymerization of DMA, AA and HEMA: To a solution of required monomers (15.2×10^{-3} mol) in dry xylene (3 mL), was added AIBN (1.54 mg, 9.38×10^{-6} mol), and the alkyne-trithiocarbonate agent **2** (0.019 g, 4.69×10^{-5} mol) and nitrogen was bubbled for 15–30 min. The reaction mixtures were allowed to warm under nitrogen atmosphere to 80 °C for 24 h. The homopolymers **4b–d** were precipitated in cold hexane and the conversion was evaluated by FTIR and NMR.

2.1.3 Click Reactions

2.1.3.1 Click Coupling between homopolymers 4a and 4b–d: Homopolymer **4a** (0.61 g, 1×10^{-4} mol) and **4b** (0.68 g, 1×10^{-4} mol) CuI (45.2 mg, 2.37×10^{-4} mol) were added and then evacuated using a Schlock line (ca. 10 min) and backfilled with nitrogen. To the resulting mixture was added a solution of degassed 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.339 g, 2.23×10^{-4} mol) in freshly distilled dry tetrahydrofuran (THF) (5 mol). The flask was placed in a constant temperature oil bath at 40 °C for 48 h. After 2 days, the solution that turned from yellow to dark brown was cooled down to room temperature. Block copolymer was obtained by precipitation in cold water. The crude product was dissolved in dichloromethane and washed two times with a saturated ammonium chloride solution and water to remove the copper salt. The organic layer was dried with magnesium sulfate. The solvent removal under reduced pressure yielding the product as a white solid which was further dried under vacuum. The resulting polymers (**5a–5c**) were evaluated by FTIR and NMR, and the peak assignments are as follows:

IR cm^{-1} : **5a**: 749, 841, 986, 1190, 1386, 1435, 1646, 1724, 2800, 2949; **5b**: 751, 834, 986, 1141, 1356, 1436, 1615, 1722, 2850, 2930; **5c**: 738, 850, 984, 1148, 1455, 1626, 1728, 2820, 2979 **¹H NMR** (CDCl_3): δ ppm: **5a**: 0.99 (t, 6H), 1.03–2.9 (m, 61H), 3.3–3.6 (m, 7H), 4.2 (t, 2H), 5.31 (s, 2H), 7.3 (s, 1H), 10.8 (s, 1H); **5b**: 0.86 (t, 6H), 1.03–2.33 (m, 58H), 3.60–3.73 (m, 7H), 4.2 (t, 2H), 5.31 (s, 2H), 7.1 (s, 1H); **5c**: 0.85 (t, 6H), 1.03–2.3 (m, 61H), 3.0–3.6 (m, 7H), 4.2 (t, 2H), 5.30 (s, 2H), 7.1 (s, 1H)

2.2 Analytical Techniques

2.2.1 Nuclear Magnetic Resonance (NMR) Spectroscopy— ^1H NMR and ^{13}C NMR spectra were recorded on a 300-MHz Bruker AC300 spectrometer using deuterated chloroform as a solvent (chloroform- d , Aldrich, 99.9%).

2.2.2 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR FT-IR)—ATR FT-IR spectra of the samples were obtained on a Perkin-Elmer Spectrum One FT-IR spectrometer using a single reflection horizontal ATR accessory. Each spectrum was collected in the range of 4000–400 cm^{-1} by cumulating 100 scans at a resolution of 4 cm^{-1} . The scan speed was set at 0.5 cm/s . Baseline was corrected for all spectra using the Perkin-Elmer Spectrum software.

2.2.3 Size Exclusion Chromatography (SEC)—The number average molecular weight (M_n) and poly dispersity index (M_w/M_n) copolymers were determined using SEC equipped with a LC 1120 HPLC pump, a MIDAS (Type 830) autosampler, a differential refractive index (DRI) detector, a 5.0 μm bead size guard column (50–7.5 mm). DMF was used as the eluent at a flow rate of 1 mL min^{-1} at 60 °C. The SEC system was calibrated with poly(styrene) standards.

2.2.4 Differential Scanning Calorimetry (DSC)—The thermal properties of copolymers were studied by differential scanning calorimetry (DSC) using a TA Instrument DSC 2010 thermal analyzer under a nitrogen atmosphere. For measurement of the glass transition temperature (T_g), 5–10 mg of sample was placed in an aluminum holder and heated from 0 to 150 °C. The heating rate was set to 2 °C min^{-1} . An empty aluminum pan was used as a reference material. The T_g value was determined from the onset, end, and inflection of a step transition using the TA Instruments Thermal Advantage Universal Analysis software. All samples were dried under vacuum at 40 °C for 24 h prior to DSC measurements

2.2.5 Ultraviolet-Visible Spectroscopy—Ultraviolet-visible (UV–Vis) spectroscopy was carried out using a Cary 300 Bio UV–Vis spectrophotometer (Varian). Absorption spectra were measured in THF from 200 to 800 nm with a resolution of 1 nm in a 10 mm UV cuvette.

2.2.6 AFM Imaging Sample Preparation—To prepare films for AFM imaging, powders of selected block copolymers (PMMA-*b*-PAA and PMMA-*b*-PHEMA) were dissolved in tetrahydrofuran (THF) at a concentration of 20 mg/mL. The resulting polymer solutions were spin-coated on silicon wafers (cut into 1 cm × 1 cm squares) at 3000 rpm for 1 min. For comparison, the homopolymers were also studied. Powders of PMMA, PAA and PHEMA were dissolved in their respective solvents. PMMA was dissolved in THF, while PAA and PHEMA were dissolved in a 95:5 (by volume) ethanol/water mixture. The solution concentrations for the homopolymers were kept at 20 mg/mL, and spin-coating on silicon wafers were performed using the same conditions as mentioned above. The thickness of these spin-coated films ranged from 50–60 nm.

For the liquid medium imaging experiments, the samples were immersed in Dulbecco's phosphate buffered saline 1× (PBS) solution (Invitrogen, Carlsbad, CA) for 1 h prior to imaging. This step was undertaken to equilibrate the film surface to the liquid environment. PBS is composed of the following electrolytes: NaCl, Na₂HPO₄, KCl, KH₂PO₄, MgCl₂ and CaCl₂.

In order to induce equilibrium chain orientation, two film annealing procedures were performed. Solvent annealing was undertaken by exposing the samples to solvent vapors for 4 h under a stream of flowing N₂ gas. A combined thermal and solvent annealing procedure was performed by placing the samples in a petri dish and adding drops of the PBS solution at areas adjacent to the samples. The dish containing the samples were then kept in an oven set at 50 °C for 1 h.

2.2.7 AFM Imaging Procedure—A Dimension 3000 AFM (Veeco, Santa Barbara, CA) was used for imaging in air. The experiments were performed in ambient air conditions, namely a temperature of 22±1 °C and 45–55% relative humidity (RH). Experiments were performed in tapping (or intermittent contact) mode in order to obtain height and phase information [38–39]. An RFESP tip (Veeco, Santa Barbara, CA) with a nominal spring constant of 3 N/m and a resonant frequency of 60–80 kHz was used. Scanning was typically performed at a rate of 0.5 Hz along the fast scan axis. Two scan sizes were used, namely 2 μm × 2 μm and 500 nm × 500 nm. The latter scan size was selected in order to obtain a closer look on the spacing and regularity of the nanostructures on the surface.

In tapping mode, the cantilever/tip assembly is sinusoidally vibrated at its resonant frequency and the sample x-y-z piezo is adjusted using feedback control in the z-direction in order to maintain a constant setpoint. The feedback signal to the z-direction sample piezo is a measure of topography. The AFM extender electronics is used to measure the phase angle (or lag) between the cantilever piezo drive signal and the cantilever response during sample engagement. This phase angle is the lag between the cantilever in free vibration and the cantilever during its interaction with the sample. If the tip is not interacting with the sample, such that the only force acting on the system is the periodic driving force, then the phase angle is zero; the angle is generally nonzero when in contact with the sample. The phase angle depends on the sample's viscoelasticity, the Young's modulus and adhesion hysteresis. It is also known to depend on the forces due to atomic interactions between the tip and sample surfaces. For a nonuniform sample, the phase contrast is a measure of sample composition [40–41]. From the applications viewpoint, it is of interest to determine the variation in morphology as a function of test temperature since this may have implications

on film stability. To this end, the samples were mounted on a Veeco Dimension Heater/Cooler stage (DMHC35–250, Veeco, Santa Barbara, CA) and phase contrast images were taken at 22, 60 and 100°C. Scanning was performed at a rate of 0.5 Hz on a $2\ \mu\text{m} \times 2\ \mu\text{m}$ area.

Friction force microscopy (FFM) was also used to image the samples on which tapping mode measurements were made. In FFM, the sample is scanned at an angle of 90° to the long axis of the cantilever in contact mode. As the friction that the tip encounters varies, the cantilever/tip assembly is twisted to a greater or lesser extent. The twist is measured by the AFM photodetector and serves as a measure of the friction force. The height data is measured simultaneously; this data is the feedback signal to the z-direction piezo [Bhushan and Qi, 2003; Scott and Bhushan, 2003]. Experiments were performed using an NP tip (Veeco, Santa Barbara, CA) with a nominal spring constant of 0.58 N/m, and a normal load of 50 nN was used and data is collected for the trace direction using a scan rate of 0.5 Hz and a scan size of $2\ \mu\text{m} \times 2\ \mu\text{m}$.

For imaging in liquid medium, a Multimode AFM (Veeco, Santa Barbara, CA) equipped with a modified tip holder was used [42]. A horizontal slot was carved out in the opening of a non-fluid Multimode tip holder in order to insert a glass slide. For these experiments, an RFESP tip with a nominal spring constant of 3 N/m was used. Imaging was performed in tapping mode at a scan rate of 0.5 Hz for two scan sizes, namely $2\ \mu\text{m} \times 2\ \mu\text{m}$ and $500\ \text{nm} \times 500\ \text{nm}$.

3. Results and Discussion

3.1 Synthesis

Figure 2 presents the strategies for the synthesis of the compounds of interest. The ‘click’ coupling between an azide functionalized poly(methyl methacrylate) homopolymer and an alkyne functionalized hydrophilic homopolymer was the methodology chosen to synthesize the block copolymers. The advantage of the ‘click’ methodology is that the PMMA block size will be consistent throughout the series of block copolymers. The goal is to compare physical and biological properties within the series, so the structures need to be as regular as possible. It is possible to synthesize block copolymers with a RAFT catalyst and sequential addition of monomers with consistent structures. However, in practice achieving exactly the same molecular weight of the methyl methacrylate block for every block is challenging. In addition, the click methodology allows for a degree of synthetic flexibility, other hydrophobic blocks could be used if it is necessary.

Synthesis of the RAFT reagent trithiocarbonate 2-decylsulfanylthiocarbonylsulfanyl-2-methyl-propionic acid **1** was prepared according to the reported procedure [36]. The azido functionalized RAFT chain transfer agents (CTAs), azido-trithiocarbonate **2** and alkynyl functionalized RAFT CTAs, alkyne-trithiocarbonate **3** have been efficiently prepared by reacting equimolar quantities of **1** with 3-azido propanol and propargylalcohol respectively in CH_2Cl_2 at room temperature. Two functionalized CTAs **2** and **3** have been used to mediate RAFT homo polymerizations of hydrophobic monomer methyl methacrylate (MMA) and hydrophilic monomers *N,N*-dimethylacrylamide (DMA), acrylic acid (AA), 2-hydroxyethyl methacrylate (HEMA) in dry xylene at $80\ ^\circ\text{C}$ by using AIBN as the initiator. The resulting azido terminated homo polymer **4a** was reacted with various acetylene terminated homopolymers **4b–d** by using CuI catalyzed click chemistry. The pure block copolymer **5a–c** thus formed were precipitated in a large excess of cold n-hexane and dried at $40\ ^\circ\text{C}$ in vacuum for 6 h. The resulting block copolymers are not water soluble, making them suitable for aqueous AFM evaluation as well as cell culture. Several reactions were

performed targeting different molecular weights using different feed ratios (1/1, 1/3, 3/1) of homopolymers **4a** to **4b–d**.

3.2 Nuclear Magnetic Resonance Spectroscopy

^1H NMR of homopolymers **4a–d** confirmed the absence of acrylic group as well as the quantitative conversion (98–100%) of the monomers. The structures of the diblock copolymers **5a–c** were confirmed by ^1H NMR spectra. NMR studies also confirmed the exclusive formation of the 1,4-cycloaddition product. Block copolymers **5a–c** showed the appearance of a singlet at 7.0 ppm, which corresponds to the triazole ring.

3.3 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR FT-IR)

FTIR spectroscopy allows for the observation of changes of functional groups during the click reaction by observing the reduction of intensity of the IR signal corresponding to the alkyne and azide groups. Typical IR stretches for alkynes and azides respectively can be found at 3300 cm^{-1} (alkyne) and 2100 cm^{-1} (azide). Figure 3 displays typical FTIR spectra of synthesized compounds **1–3** with **2** displaying the azide stretch and **3** the alkyne stretch. Homopolymers with azide **4a** and alkyne **4b** groups and block copolymers **5a–c** without the azide or alkyne stretches are also shown. The IR stretches at 2150 cm^{-1} , 3320 cm^{-1} found in **4a** and **4b** respectively confirmed the existence of azide and acetylene groups even after the polymerization. The successful coupling of the homopolymers **5a–c** was evidenced by the disappearance of the strong azide signal at 2150 cm^{-1} and alkyne signal at 3320 cm^{-1} which were replaced by triazole stretches at 800, 1650, and 2800 cm^{-1} .

3.4 Size Exclusion Chromatography

The homopolymers **4a–d** synthesized from the initiators **2** and **3** had the expected number average molecular weight (M_n) exhibiting a narrow molecular weight distribution (MWD), as shown in the polydispersity index (PDI) summary in Table 1. The click reaction between **4a** and **4b–d** was carried out with the choice of various molar ratios of homopolymers and it is of interest to examine the PDI of the block copolymers as well. Figure 4 shows that the resultant block copolymers have PDI close to unity, indicating successful synthesis. SEC was also used to determine the molecular weight distribution of the homopolymers and block copolymers, which is presented in Figure 5. As expected, the lighter homopolymers (dashed and dashed/dotted curves) eluted later than the heavier block copolymer (solid curve). The formation of well-defined products with mono modal molecular weight distributions was confirmed using SEC. As depicted in Figure 5 and summarized in Table 1, the click reaction of between the azido functionalized homo polymer and the acetylene functionalized homopolymers resulted in a molecular weight shift. The molecular weight shift and the lack of a peak corresponding to either homopolymer indicates that the click reaction is complete and quantitative. The results of SEC analysis also implied that the block copolymers **5a–c** were synthesized with controlled polymerization and narrow molecular weight distribution having molecular weights in the range of $5\text{--}14 \times 10^3\text{ g/mol}$.

3.5 Differential scanning calorimetric (DSC) studies

The glass-transition temperatures (T_g) shown in Table 1 also effectively confirmed the homopolymerization of the corresponding monomers. The homopolymers **4a–d** exhibited T_g values at 103, 115, 110, 82, 48 °C, respectively, as shown in Table 1. The T_g of the block copolymers **5a–c** obtained using DSC confirmed the presence of the two polymer blocks. T_g of the block copolymers **5a–c** was observed at 103, 80 and 45 °C in addition to the T_g of PMMA. Melting of HEMA, AA and DMA was not observed in the block copolymer, which indicated their amorphous morphology.

3.6 UV–Vis Photo spectroscopy

Further analysis was carried out with homopolymers **4a–d** as well as the click reaction products, block copolymers **5a–c** by employing UV–Vis spectroscopy in THF. The goal is to detect chemical groups present in the block copolymers but not in the homopolymers. The spectra of the purified block copolymers **5a–c** as well as homopolymers **4a–d** is shown in Figure 6. While the reactants (**4a–d**) did not show significant absorptions above a wavelength of 250 nm, the resulting block copolymers (all molar ratios) showed absorption maximum at 260 nm due to the formation of triazole unit.

3.7 Micelles of PMMA-b-PAA, PMMA-b-PDMA, PMMA-b-PHEMA diblock Copolymers

As the diblock copolymers **5a–c** are amphiphilic in nature, they could undergo micellization in appropriate solvents. The formation of micelles would suggest that these polymers have the potential to phase separate in the solid state. Attempts were made to form micelles using three amphiphilic diblock copolymers **5a–c** in water. The block copolymer with 1/1 composition of (PMMA: PAA, PMMA: PDMA, PMMA: PHEMA) did not dissolve in water directly. However, the micellization was induced from the solutions of **5a–c** through slow titration into a non-solvent. Accordingly, **5a–c** was dissolved in a small amount of N-methylpyrrolidinone (NMP), which is a good solvent for both the hydrophilic and hydrophobic segments of block copolymer and this solution was gradually diluted with the addition of water, a selective solvent for hydrophilic segment. Upon addition of water, the solution developed a blue tint indicative of the formation of micelles. Dynamic light scattering measurements of the solution showed the presence of micelles with hydrodynamic diameter of 255, 223.6 and 82.3 nm at 25 °C in a water/NMP (9/1, v/v) system. The size of the micelles was observed at different angles and found that there was no angular dependence. The particle size distribution of micelles is shown in Figure 7, a unimodal size distribution for polymer **5a–c** was observed. The size of most micelles formed from polymer **5c** is smaller than polymer **5a** and **5b** and can be easily understood as a more compact core is formed from polymer **5c**.

As the thiocarbonylthio groups present in the initial RAFT agents has been retained in the reactions the polymeric products could be used as macro RAFT agents. Reports about the nucleophilic substitution of thiocarbonylthio groups by various nucleophiles and ionic reducing agents to generate thiols also have emerged. The complete desulfurization can also be achieved by either thermolysis or radical-induced reactions [43]. Based on these reports the synthesized block copolymers could also be transformed into a range of useful compounds as it incorporates terminal thiocarbonylthio groups for further modifications. In addition, the synthesized block copolymers **5a–c** could also be further modified to well-defined end functional block copolymers [44–45].

3.8 AFM Imaging

The synthesized block copolymer systems have not been previously imaged by AFM to characterize the morphology. Two of the block copolymer systems, MMA-AA (1/1 molar ratio) and MMA-HEMA (1/1 molar ratio) were chosen so that the sample preparation and imaging conditions can be determined.

3.8.1 Imaging in ambient conditions—Height and phase images taken in ambient air conditions on the block copolymers and homopolymers after solvent annealing are summarized in Figure 8. The height images show that the block copolymers have more topographic variation compared to the homopolymers. This is likely to occur since the homopolymers are amorphous and are not expected to adopt a particular configuration that may lead to a large topographic variation. However, height variation does not identify between the two components of the block copolymer systems (i.e., MMA vs. AA or MMA

vs. HEMA). This information could be obtained from the phase images. From the $2\ \mu\text{m} \times 2\ \mu\text{m}$ images, light and dark regions are observed, which correspond to high phase lag (AA or HEMA) and low phase lag (MMA) regions, respectively. The hydrophilic polymers (AA or HEMA) are expected to exhibit higher phase lag compared to the hydrophobic polymer (PMMA) since absorption of water from the ambient environment could soften the former and make them more viscoelastic than the latter. The presence of the two phases is further confirmed in the $500\ \text{nm} \times 500\ \text{nm}$ images, where spherical features on the order of 10–30 nm in diameter are seen. These features are evidence that these block copolymer systems adopt either cylindrical or bicontinuous microstructure [14]. Therefore, we have shown through the phase images that there is a significant difference in the viscoelastic properties, elastic modulus and the adhesion properties of the polymer components. This implies that the phase contrast denotes the location of the polymer domains. As mentioned earlier, morphological variation at elevated test temperatures is of interest as this environment could have implications on the stability of the films. It should be noted that the morphology at elevated temperatures (60 and 100 °C) did not exhibit any significant change compared to ambient temperature morphology (data not shown). This is because the glass transition temperatures (T_g) of the component blocks are within a few degrees of each other, i.e., 105 °C (PMMA), 103 °C (PAA) and 101 °C (PHEMA) [46–48], so each component is affected by the environment evenly.

Figure 9 is a summary of the friction images taken in ambient air. The components of the block copolymers exhibited a slight difference in their friction behavior, even though they showed significant difference in their viscoelastic properties. This is because at room temperature, all polymer components (MMA, AA, HEMA) are well below their glass transition temperatures (T_g) [47]. As a result, the two components exhibit similar glassy behavior and thus, their friction properties are comparable.

3.8.2 Imaging in liquid medium and water absorption of the AA and HEMA blocks

The block copolymer and homopolymer surfaces were imaged while immersed in phosphate buffered saline (PBS) in order to simulate physiological conditions. Prior to imaging, the samples were subjected to the combined thermal and solvent anneal procedure, as described in Section 2.2.6. Figure 10 shows height and phase images, where it can be seen that there is a distinct phase contrast due to differential water absorption between the individual components. In the phase images of the block copolymers, the dark areas correspond to the MMA domains, while the light areas correspond to the AA and HEMA domains. The presence of surface features such as protrusions and pits on the block copolymers are comparable to features on other nanostructured surfaces reported to exhibit favorable protein or cell adhesion and growth [8–10].

Even though the stoichiometric proportion between the two blocks is 50/50, this is not reflected in the images, where it appears as if the AA and HEMA were present in greater amounts. This is interpreted as a consequence of chain swelling, as hydrophilic polymers, are known to absorb water and swell. This phenomenon is well-documented for polymers containing acrylic acid and 2-hydroxyethyl methacrylate [49–54]. Very low phase contrast is seen for the homopolymers, as expected. It should be noted that PAA was not included in Figure 10 because its films were observed to immediately dissolve in PBS solution.

Aside from swelling, a chemical reaction also takes place in the polyacrylic acid chains. PAA is a weak acid with a pK_a of 4.7 [55]. Since the PBS solution has a pH of 7.4, this implies that the acrylic acid chains will ionize while the PMMA-b-PAA film is immersed in PBS, and the PAA block is a polyanion in physiological conditions. This scenario is shown in Figure 11, where Na^+ is one of the possible counterions. The cations of the other salts in PBS solution, such as K^+ , Ca^{2+} and Mg^{2+} can also act as the counterion.

It has been shown on other hydrophilic polymer systems that swelling increases as the number of –COOH groups on the polymer backbone increases as a consequence of hydrogen bonding interactions [56]. Since the carboxylic acid groups have been ionized, the swelling in the PAA could be suppressed, implying that the PMMA-b-PHEMA surface could have a greater tendency to swell relative to PMMA-b-PAA. This accounts for the prevalence of protruded regions in the PMMA-b-PHEMA surface, which is interpreted as areas experiencing a greater extent of swelling.

In this study, we have demonstrated the variations in the morphology of these block copolymer systems in air and while immersed in physiological buffer medium. The key results and images obtained will serve as a useful guide in the design of AFM-based protein adhesion studies using the block copolymer systems that were analyzed in this investigation.

4. Conclusions

Two RAFT CTAs with azido and alkynyl containing groups were synthesized. The radical homo polymerizations of monomers MMA, AA, HEMA and DMA mediated by RAFT agents in a controlled way with predictable and narrow molecular weight distributions were investigated. The clickable moieties of the RAFT CTAs were well preserved during the RAFT polymerization of homopolymers in accordance to the FTIR data. The synthesis of a novel class of block copolymers with various molar ratios through click coupling of homopolymers of MMA blocks with azide functionality and AA, DMA, HEMA blocks with acetylene functionality has been accomplished. Light scattering from NMP/water solutions of these polymers showed that the original polymers were fully dissolved and the molecular weight of the polymer did affect the size of micelle particle size.

Based on AFM imaging experiments, solvent annealing successfully induced phase contrast corresponding to the polymer domains in the block copolymers. The phase images confirm that there is a significant difference in the viscoelastic properties between methyl methacrylate and acrylic acid, as well as between methyl methacrylate and 2-hydroxyethyl methacrylate. However, only a slight difference in the friction characteristics was observed as all polymer components are below their T_g values.

Imaging in physiological buffer medium reveals phase contrast on the block copolymer systems resulting from differential water absorption between the individual components. A greater amount of the hydrophilic chain portions corresponding to acrylic acid and 2-hydroxyethyl methacrylate was observed on the surface as a result of water uptake and swelling of these chain portions. The distribution of the nanoscale features observed in these block copolymers are comparable to those on other surfaces reported to exhibit favorable protein or cell adhesion and growth. These results obtained from this study will be valuable for future protein and cell adhesion experiments using these block copolymer systems.

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Fig. 1. Schematic showing the application of the scaffold material on the damaged alveolar bone. Inset shows nanoroughness on the scaffold surface where the cells adhere to, which simulates the natural surface.

Fig. 2.

Synthesis of block copolymers through RAFT and click chemistry.

Reagents and conditions: i) a. 3-azido propanol, EDC, DCM, 25–30 °C, 6–10 h, yield 40–65%; ii) propargylalcohol, EDC, DCM, 25–30 °C, 6–10 h, 65%; iii) MMA, AIBN, xylene, 80 °C, 24 h, 58–78%; iv) AA/DMA/HEMA, AIBN, xylene, 80 °C, 24 h, 58–78%; v) 4a,4b/4a,4c/4a,4d, CuI, DBU, THF, 40 °C, 48 h, 58–78%.

Fig. 3.
Attenuated total reflectance Fourier transform infrared(ATR FT-IR) analysis of the compounds **1**, **2**, **3**, **4a–b** and **5a–c** (from bottom- top).

Fig. 4.
Polydispersity index(PDI) of block copolymers with different molar ratios of homopolymers.

SEC showing molecular weight distribution of homopolymers and block copolymers

Fig. 5. Representative size exclusion chromatogram (SEC) showing molecular weight distribution of homopolymers and block copolymers.

Fig. 6.
Representative UV-Vis spectra of **4a**, **4b-d** and **5a-c** in THF.

Fig. 7. Formation of small unilamellar vesicle (SUV) was confirmed for all three block copolymers **5a–c** with 1/1 ratio. Particle sizes were measured by photon correlation method. Numbers shown are the mean particle size (standard deviation – volume weighted) of the individual samples.

Fig. 8. Height and phase images obtained using tapping mode in ambient air of samples subjected to solvent annealing. In the phase images of the block copolymers, the dark areas correspond to the MMA domains, while the light areas correspond to either AA or HEMA domains.

Fig. 9.

Height and friction images obtained using contact mode in ambient air of samples subjected to solvent annealing. A low friction contrast between the polymer domains is observed in the block copolymers.

Fig. 10.

Height and phase images obtained using tapping mode with the samples immersed in PBS solution. In the phase images of the block copolymers, the dark areas correspond to the MMA domains, while the light areas correspond to either AA or HEMA domains. AA and HEMA are predominant due to water absorption and swelling of these components.

Fig. 11. Ionization of the acrylic acid groups in the PMMA-b-PAA block copolymer after immersion in PBS solution. The other cations in PBS (such as K^+ , Ca^{2+} and Mg^{2+}) can also act as the counterion.

Table 1

Representative SEC and DSC data for homopolymers and block copolymers (1/1 ratio) synthesized through RAFT and Click chemistry.

Sample No.	Polymer	^a M _w (kg/mol)	Poly dispersity index (PDI)	DSC T _g (° C)
1	4a	6.4	1.03	103, 115
2	4b	6.8	1.20	110
3	4c	5.9	1.10	82
4	4d	8.1	1.06	48
5	5a	12.2	1.10	103, 115
6	5b	11.7	1.23	80, 98
7	5c	14.3	1.09	45, 105

^a Measured by gel permeation chromatography (GPC) in N,N-dimethylformamide at 60°C