Published in final edited form as:

Macromolecules. 2012 November 27; 45(22): . doi:10.1021/ma301556v.

Surface-initiated Polymerization of Azidopropyl Methacrylate and its Film Elaboration via Click Chemistry

Sampa Saha, Merlin L. Bruening*, and Gregory L. Baker*

Department of Chemistry, Michigan State University, East Lansing, MI 48824, USA

Abstract

Azidopropyl methacrylate (AzPMA), a functional monomer with a pendent azido group, polymerizes from surfaces and provides polymer brushes amenable to subsequent elaboration via click chemistry. In DMF at 50 °C, click reactions between poly(AzPMA) brushes and an alkynylated dye proceed with >90% conversion in a few minutes. However, in aqueous solutions, reaction with an alkyne-containing poly(ethylene glycol) methyl ether (mPEG, $M_n=5000$) gives <10% conversion after a 12-h reaction at room temperature. Formation of copolymers with AzPMA and polyethylene glycol methyl ether methacrylate (mPEGMA) enables control over the hydrophilicity and functional group density in the copolymer to increase the yield of aqueous click reactions. The copolymers show reaction efficiencies as high as 60%. These studies suggest that for aqueous applications such as bioconjugation via click chemistry, control over brush hydrophilicity is vital.

Keywords

ATRP; click chemistry; copolymer brush; surface-initiated polymerization; post functionalization; hydrophilic; PEG chain

Introduction

The application of controlled radical polymerization techniques to surface-initiated polymerizations provides control over polymer film thickness and structure.¹⁻⁵ Recent studies have exploited surface-initiated polymerizations to obtain functional brushes, including brushes that segregate into different domains,^{6,7} capture specific biomolecules,^{8,9} create responsive surfaces that undergo temperature-driven changes in solubility (e.g. lower critical solution temperature (LCST) behavior), and modify other properties.^{10-12,13} The ideal route to functional polymer brushes includes the synthesis of monomers with requisite functionalities and subsequent surface-initiated polymerization. However, the synthesis of complex functional monomers can be difficult, and some functional groups are incompatible with the polymerization conditions. An alternative strategy employs post-polymerization modification of poly(2-hydroxyethyl methacrylate), poly(acrylic acid), poly(glycidyl methacrylate), and other brushes.³ While straight-forward, this approach often requires several chemical steps, and the derivatization reactions may not be quantitative.

“Click” chemistries,¹⁴⁻¹⁶ such as the Cu(I)-catalyzed azide–alkyne cycloaddition (the CuAAC ‘click’ reaction), the thio-ene and thio-yne reaction,¹⁷ and others are versatile

*Corresponding Author Gregory L. Baker, bakerg@msu.edu; 517-355-9715; Merlin L. Bruening bruening@chemistry.msu.edu, 517-355-9715;.

Supporting Information. Figures SI-1 – SI-11. This material is available free of charge via the Internet at <http://pubs.acs.org>.

methods for post-polymerization modification of polymers^{14,18-28} and surfaces.¹⁸⁻²⁷ The CuAAC reaction, which is highly selective and broadly tolerant to functional groups, often proceeds in near-quantitative yields. Thus, combining surface-initiated polymerization and subsequent click reactions is a powerful strategy for synthesis of highly functionalized polymers. Azide-containing brushes, as homopolymers or copolymers, can serve as a “platform” for elaboration of a variety of functional polymer brushes in a single reaction.

Copolymerization of a clickable monomer with appropriate comonomers should provide control over both the “click” site density, and importantly, the solubility of the brush. In this report, we focus on modifying brushes with CuAAC click reactions, which require monomers that contain alkynes or azides,²⁸⁻³¹ or formation of alkynes and azides via a post-polymerization modification.³²⁻³⁵ There are a few examples of clickable monomers, polymerized by surface-initiated polymerization, and then modified by the CuAAC reaction.³⁶⁻³⁹ In addition, Patton and coworkers showed that alkynylated polymer brushes used in the CuAAC reaction also support thio-yne click chemistry.^{40,41} Also, several groups anchored glycidyl methacrylate polymers to surfaces, enabling installation of clickable groups in thin polymer films.^{35,42}

Matyjaszewski et al.²⁸ and DuBois et al.²⁹ polymerized 3-azidopropyl methacrylate (AzPMA) and used click chemistry to modify the side chains of the resulting poly(3-azidopropyl methacrylate) (poly(AzPMA)). Importantly, both groups found that the Cu-based Atom transfer radical polymerization (ATRP) catalyst did not initiate side reactions with the azides.⁴³ Others have polymerized AzPMA. Crownover *et al.* used RAFT (Reversible addition–fragmentation chain-transfer) to synthesize poly(AzPMA) for drug delivery,⁴⁴ and Hu et al. copolymerized AzPMA with poly(oligo(ethylene glycol) monomethyl ether methacrylate), also for drug delivery. Ricardo *et al.* used AzPMA to synthesize “clicked” monomers.⁴⁵

Other poly(azidoalkyl methacrylate)s have been reported. Xu and coworkers polymerized 2-azidoethyl methacrylate from Au substrates and clicked ethynyl ferrocene to the poly(2-azidoethyl methacrylate) brush.³⁶ In their modification of carbon nanotubes, Zhang *et al.* bound poly(2-azidoethyl methacrylate) to the nanotubes and then clicked poly(propargyl methacrylate) to the nanotubes.^{46,47} Talelli et al. copolymerized 2-azidoethyl methacrylate with 2-hydroxypropyl methacrylate⁴⁸ and Canalle et al. copolymerized 2-azidoethyl methacrylate with methyl methacrylate, and anchored the copolymer on an alkynylated surface.⁴⁹ The same group copolymerized 2-azidopropyl methacrylate with 2-hydroxypropyl methacrylate.⁵⁰ Benicewicz used RAFT to grow 6-azidohexyl methacrylate from silica nanoparticles; small molecules and polystyrene were appended to the poly(6-azidohexyl methacrylate) by the CuAAC reaction.⁵¹ Mespouille et al. synthesized 2-(2-azidoethoxy)ethyl methacrylate, which is more hydrophilic than azidoalkyl methacrylates.⁵²

Alternately, -alkynyl groups can be incorporated into polymers by polymerization. Usually, -alkynyl monomers are polymerized as their trimethylsilyl-protected derivatives to avoid potential side reactions, and deprotection after polymerization makes the alkynes available for click reactions.⁵³⁻⁵⁵ However, Song et al. successfully polymerized 2-propargyl methacrylate from silica without protecting the alkynyl group,³⁸ and Cai reported similar results for propargyl methacrylate grown from PVDF membranes.³⁹

This paper focuses on developing aqueous Cu-catalyzed click reactions for brush modification. We developed conditions for growing thick poly(AzPMA) brushes and copolymer brushes prepared with AzPMA and ethylene glycol methyl ether methacrylate (mEGMA) or polyethylene glycol methyl ether methacrylate (mPEGMA). During copolymerization, we found that these monomers enter into brushes nearly randomly,

resulting in a uniform distribution of the azide groups.^{10,56} Varying the fraction of EGMA and mPEGMA in the brushes controls the hydrophilicity of AzPMA copolymers and enables attachment of water soluble molecules, e.g. biological molecules, as exemplified by functionalization of brushes with dyes and water-soluble polymers.

Experimental Section

Materials

Unless otherwise noted, all chemicals were obtained from Sigma-Aldrich. Fluorescein, polyethylene glycol monomethyl ether (mPEG, $M_n = 5000$ g/mol), sodium hydride, propargyl bromide (80% solution in toluene), 11-mercapto-1-undecanol (MUD, 97%), 2-bromopropionyl bromide (2-BPB, 97%), anisole (99.7%), *N,N*-dimethylformamide (DMF, 99.8%), CuBr (99.999%), CuBr₂ (99.999%), Me₄Cyclam (99%) 4,4'-dinonyl-2,2'-bipyridyl (dnNbpy, 97%) and pentamethyldiethylene triamine (PMDETA, 97%) were used as received. 2,2'-Bipyridine (bpy, 99%) was recrystallized from hexanes and sublimed prior to use. Triethylamine was distilled from calcium hydride under an argon atmosphere at reduced pressure. Azidopropyl methacrylate (AzPMA),²⁸ the alkynylated fluorescein methyl ester,⁵⁷ and alkynylated mPEG ($M_n \sim 5000$)⁵⁸ were synthesized by published procedures. The purity of monomer and alkyne derivatives were assayed by ¹H and ¹³C NMR spectroscopy, obtained at room temperature using a Varian UnityPlus-500 spectrometer at 500 and 125 MHz, respectively, with the chemical shifts reported in ppm and referenced to signals from residual protons in the solvent. Ethylene glycol methyl ether methacrylate (mEGMA, 99%) and poly(oligoethylene glycol methyl ether methacrylate) (mPEGMA, $M_n \sim 300$, 98%) were passed through a 10 cm column of basic alumina to remove inhibitors. After purification, monomers and solvents were transferred to Schlenk flasks, de-gassed using three freeze-pump-thaw cycles and then transferred into a drybox. The process of immobilizing initiators on ITO and gold substrates is described elsewhere.⁵⁹⁻⁶¹

Homo and Copolymerization of AzPMA, mEGMA and mPEGMA from initiators immobilized on Au substrates

AzPMA was polymerized in a N₂-filled drybox. CuBr (6 mg, 0.04 mmol), CuBr₂ (5 mg, 0.02 mmol), Me₄Cyclam (10 mg, 0.04 mmol), and dnNbpy (16 mg, 0.04 mmol) were added to a round bottom flask containing a 20 mL solution of monomer in DMF/anisole (AzPMA/DMF/anisole = 2:1:1 v:v:v, [AzPMA] = 3 M). The well-stirred mixture was heated in an oil bath at 50 °C until the solution turned light green, and then the solution was transferred into vials containing initiator-modified Au or ITO substrates (50 °C) to start surface-initiated polymerization. At predetermined reaction times, substrates were removed from vials, washed sequentially with ethyl acetate and THF, and then dried under a flow of N₂ in a drybox. The same conditions were used to polymerize monomers with PMDETA- and bpy-based copper catalysts. The [monomer]:[Cu(I)]:[ligand] was 300:1:1.1 in all cases, except for three trial AzPMA polymerizations (Figure 1), CuCl/PMDETA/CuBr₂/DMF at 50 °C, CuCl/bpy/CuBr₂/isopropanol at 50 °C, and CuCl/bpy/CuBr₂/isopropanol at RT. For these polymerizations the [monomer]:[Cu(I)]:[Cu(II)]:[ligand] was 300:1:0.1:1.1 for the PMDETA system and 300:1:0.1:2.4 for the bpy system. In all polymerizations the monomer:solvent was constant at 1:1, but the ratio of monomers was varied in copolymerizations.

Click functionalization of homo and copolymer brushes

For clicking alkynylated fluorescein to poly(AzPMA)-coated substrates, in a dry box gold-coated (or ITO) substrates modified with AzPMA homopolymer or copolymer brushes were transferred to vials containing DMF solutions (5 mL) of alkynylated fluorescein (97 mg, 0.25 mmol), CuBr (9.0 mg, 0.063 mmol) and PMDETA (13 µL, 63 µmol) at 50 °C. After a

set reaction time, substrates were rinsed with DMF and THF to remove unreacted dye, sonicated in THF for 1 min, and again rinsed with THF. The rinsed films were dried in a stream of nitrogen.

A N₂-filled glove bag was used for aqueous click modification of polymer brushes. Substrates were immersed in degassed solutions (5 mL) of alkynylated mPEG (0.252 g), CuBr (1.8 mg), and bpy (4 mg) in deionized water (Milli-Q, 18.2 MΩ cm). After 12 hours at room temperature, the substrates were rinsed with deionized water and dried in a stream of N₂.

Characterization Methods

Film thicknesses were measured using a rotating analyzer ellipsometer (model M-44; J. A. Woollam) at an incident angle of 75°. The data were analyzed using WVASE32 software, and thickness determinations were performed on at least three spots on each substrate. The refractive indices of films were assumed to be 1.5. Reflectance FTIR spectroscopy was performed using a Nicolet Magna-IR 560 spectrometer containing a PIKE grazing angle (80°) attachment. UV-vis spectra were obtained with a Perkin Elmer lambda 400 spectrometer. Atomic force microscopy (AFM) images were obtained in tapping mode with Multimode AFM and NanoScope IV software (Digital Instruments, Santa Barbara, CA) at room temperature. A tapping mode probe (NSC15) with a nominal frequency of 300 kHz was used for all experiments. Fluorescence images were collected with an Olympus FluoView FV1000 Laser Scanning Confocal Microscope (Tokyo, Japan). The images were collected using the 20× UPlanFLN (NA0.5) objectives with a 3× optical zoom. The fluorescence was excited using the 488 nm line of an Ar laser, and the emission was collected using a 535-565 nm band-pass filter.

Results and Discussion

Growth of uniform poly(AzPMA) brushes from initiators on gold

This study aims to (a) define conditions for controlled surface-initiated ATRP of poly(AzPMA), (b) vary the fraction of azides in the brush through copolymerization, and (c) tune brush hydrophilicity to enable aqueous click chemistry. Scheme I shows the synthetic route to methacrylate copolymer brushes with pendant azides and their modification by click chemistry. We established appropriate conditions for homopolymerization of AzPMA from initiators on gold by examining film growth rates for several catalyst systems (Figure 1). Ideally, film thickness should increase linearly with polymerization time, indicating a low concentration of active radicals and minimal termination. However, there is often a trade-off between control and growth rate since low radical concentrations also result in low growth rates. CuBr/Me₄Cyclam/dnNbpy catalysts typically provide rapid polymerization of methacrylate monomers and thick films on substrates. However, with AzPMA, the Me₄Cyclam/dnNbpy-based catalyst gives rapid initial film growth (X's, Figure 1) followed by a decline in the growth rate, suggesting significant termination as a consequence of a high radical concentration.

In solution polymerizations of AzPMA, PMDETA/CuCl and bpy/CuBr catalysts provide significant control over the molecular weight distribution and retention of the azide functionality.^{28,62} Compared to the Me₄Cyclam/dnNbpy system, which shows significant termination, the PMDETA/CuCl catalyst provides well-controlled polymerization of AzPMA as indicated by a nearly linear increase in thickness with polymerization time over 4 h (Figure 1, squares). (The non-zero y-intercept suggests a high initial growth rate immediately following initiation.) After 4 hours, the polymerization with the PMDETA/CuCl catalyst slowed, and the film thickness reached 260-300 nm after 12 hours (data not

shown). Because PMDETA Cu complexes are highly active catalysts for solution ATRP,^{63,64} we added 10% CuBr₂ to the CuCl/PMDETA catalyst system to improve control. However, the polymerizations were sluggish, and had non-zero y-intercepts (diamonds, Figure 1). We also explored bpy as the catalyst ligand. Surface-initiated AzPMA polymerizations yielded ~100 nm thick poly(AzPMA) films after 4 hours of polymerization at 50 °C (Figure 1, triangles), compared to ~200 nm thick brushes obtained under similar conditions with PMDETA as the ligand. Room temperature polymerization was even slower with the bpy system. The CuCl/PMDETA catalyst system provides reasonable growth rates with modest termination over several hours, and we used this system exclusively in subsequent experiments.

Reflectance FTIR spectra confirm the formation of poly(AzPMA) films. Strong carbonyl and azide bands at 1740 and 2150 cm⁻¹ reflect the growth of poly(AzPMA) brushes (Figure SI-1), and their increasing intensities with polymerization time are consistent with the thickness data in Figure 1. Also, the topographical AFM image of a 250 nm thick poly(AzPMA) brush (Figure SI-2) was smooth and uniform with an rms roughness < 2 nm (< 1% of the film thickness).

We also examined surface-initiated homopolymerization of mEGMA on gold. The CuCl/PMDETA system provided control over mEGMA polymerizations for 2 h, whereas the CuBr/Me₄Cyclam/dnNBpy catalyst gave higher growth rates but less control (Figure SI-3). Because of its good control and comparable polymerization rates for AzPMA and mEGMA, we selected the CuCl/PMDETA system for copolymerization studies.

Surface-initiated polymerization of AzPMA-co-mEGMA and AzPMA-co-mPEGMA

We polymerized poly(AzPMA-co-mEGMA) films with various monomer ratios. The ellipsometric thicknesses of the films differed by less than a factor of 2 at identical polymerization times (Figure 2), suggesting that the reactivities of AzPMA and mEGMA are comparable and consistent with a random copolymerization. We estimated the copolymer composition from reflectance FTIR spectra (Figure 3) by integrating the azide band from AzPMA and the combined carbonyl bands from mEGMA and AzPMA. We assumed that in the copolymer, the ratio of the areas of azide and carbonyl bands are proportional to the fraction of the AzPMA in the copolymer. The calculated copolymer compositions correlate directly with the ratios of the monomers in the polymerization solution (Figure 4), again suggesting a random copolymer brush.

We also copolymerized AzPMA with mPEGMA since the poly(ethylene glycol) side chain of mPEGMA (average monomer molecular weight of ~300 Da) should increase the hydrophilicity of brushes. Because of the large difference in side chain lengths in AzPMA and mPEGMA, we assessed the randomness of copolymerizations using reflectance FTIR spectroscopy. Initially, we coated several gold substrates with poly(AzPMA-co-mPEGMA) ([AzPMA]=[mPEGMA] in the polymerization solution) using polymerization times from 5 min to 4 h. The mole fraction of AzPMA in each copolymer, determined from the peak area ratios of the azide and carbonyl bands (Figure SI-4), was 0.50 ± 0.02 . We then synthesized poly(AzPMA-co-mPEGMA) brushes using different monomer ratios and characterized these copolymer films by ellipsometry (Figure SI-5) and FTIR spectroscopy (Figures SI-6). Analysis of the FTIR data for the copolymer brushes (Figure 5) indicates that the copolymer compositions approximately match the monomer ratios, as expected for a random copolymerization.

Derivatization of copolymer brushes in DMF

Surface-grafted poly(AzPMA) and AzPMA copolymers should provide excellent polymer films for elaboration via click chemistry. The random incorporation of AzPMA and mPEGMA or mEGMA comonomers into the brushes affords control over the density of azide groups, and avoids high azide concentrations that might reduce the efficiency of click functionalization.²⁸ Moreover, the high hydrophilicity provided by incorporation of mPEGMA and mEGMA into AzPMA copolymers should enhance the rate of click chemistry in aqueous media.

To assess the click reaction rate and efficiency for grafted copolymer brushes, we appended alkynyl-modified fluorescein to AzPMA homopolymers and copolymers in DMF. Both gold- and ITO-coated surfaces served as substrates; gold for analysis by reflectance FTIR, and ITO for UV-vis spectroscopy. Figure 6 shows UV-vis spectra of ~180 nm-thick poly(AzPMA-*co*-mPEGMA) (50/50) brushes on ITO after reaction with the alkynylated dye for different times. The click reaction was almost complete within 5 min, as the absorbance showed marginal increases for reaction times from 5 min to 1 hour.

To quantify the click reaction kinetics, we monitored the disappearance of the azide peak (~2100 cm⁻¹) and the appearance of the 1,2,3 triazole absorbance (C=C stretching at ~1600 cm⁻¹) in reflectance FTIR spectra taken during the first hour of the reaction (see Figure SI-7 and Figure SI-8). The click reaction was fast; 70-90% of the azides reacted within 1 min in both poly(AzPMA) and its copolymers, with higher conversions for copolymers with low azide contents. Compared to analogous click reactions in solution, click reactions may accelerate in polymer brushes. Benicewicz reported accelerated click reactions in poly(azidohexyl methacrylate) grown from silica nanoparticles,⁵¹ and Matyjaszewski observed faster click reactions in poly(AzPMA) solutions than AzPMA solutions.²⁸ Factors that may favor rapid click reactions in polymer brushes include high local concentrations of azide groups, and the 1,2,3-triazoles⁶⁵ bind Cu(I) ions and enhance the local concentration of the copper catalyst.²⁸

We used the absorbance values at $\lambda_{\max}$ to determine the amount of fluorescein dye bound to copolymer brushes. UV-visible spectra of the dye in polymer brushes and in various solvents showed essentially identical profiles so we assumed that the molar extinction coefficients for the dye in solution and on surfaces are identical. The Beer-Lambert law expressed as $A = \epsilon \lambda_{\max} \Gamma$, where A is absorbance, ϵ is the extinction coefficient in (cm³/(moles cm)), and Γ is the dye surface coverage (moles/cm²), describes the absorbance due to dye in the film. To calculate Γ , we determined the dye's molar extinction coefficient ($\epsilon = 52200$ cm²/mol) at $\lambda_{\max}$ from dye solutions in THF (Figure SI-9).

Figure 7 shows both the absorbance at $\lambda_{\max}$ and the calculated dye surface coverage in poly(AzPMA), poly(AzPMA-*co*-mPEGMA), and poly(mPEGMA) brushes grown from ITO substrates and derivatized with dye in a 5 min click reaction. The dye bound in the films was proportional to the fraction of AzPMA in the polymer brush. However, both poly(AzPMA-*co*-mPEGMA) (75/25) and poly(AzPMA) films bound ~7 g/cm² dye, suggesting a steric limit resulting from saturating the polymer film with dye, or a dense surface dye layer that hinders dye transport into the film. The data in Figure SI-8 show that the conversion was highest for the copolymers with the lowest azide content, presumably because they avoid steric effects in films.⁵¹

Figure 8 shows fluorescence microscopy images from ITO coated with ~180 nm of poly(AzPMA-*co*-mEGMA) (1:1) after a 5 min click reaction with the alkynylated dye, and a control ITO surface, treated identically but without the copper catalyst. The fluorescence of the clicked surface confirms that the dye was bound to the surface, while the lack of

fluorescence for the control surface shows that physical adsorption of the fluorescent dye to the surface is small, as Hvilsted and coworkers observed for conducting polymer films under comparable click conditions.⁵⁷

Derivatization of copolymer brushes via click chemistry with a water soluble polymer

The previous data show that when the solvent is DMF, alkynylated fluorescein is rapidly bound in poly(AzPMA) and AzPMA copolymers with mEGMA and mPEGMA. However, tethering biomolecules to brushes requires an aqueous environment, and -alkynylated mPEG 5000, a water soluble polymer, served as the model reactant. These experiments were conducted in a glove bag and at ambient temperature (23 °C), and the water-soluble catalyst was CuBr/bpy, which may be less efficient than the CuBr/PMDETA complex.⁶⁶

Poly(AzPMA) is hydrophobic and shows minimal swelling in water, and poly(AzPMA-*co*-mEGMA) films (shorter ethylene oxide chain, $p = 1$, see Scheme I) were marginally more hydrophilic than poly(AzPMA); aqueous swelling of all poly(AzPMA-*co*-mEGMA) films was less than 15% (data not shown here). Thus, the longer ethylene oxide chains in poly(AzPMA-*co*-mPEGMA) films ($p \sim 4$, see Scheme I) are critical to make the polymers sufficiently hydrophilic to allow immobilization of water soluble polymers by the click reaction.

Compared to click reactions with the alkynylated dye, the reaction of the alkynylated mPEG polymer was slow, especially for copolymers with high AzPMA content. Figure SI-11 shows reflectance FTIR spectra for ~ 50 nm thick films of poly(AzPMA) and poly(AzPMA-*co*-mPEGMA) before and after 12-h click reactions with alkynylated mPEG 5000. Integrating the azide peaks at ~ 2100 cm^{-1} in FTIR spectra before and after the click reactions reveals that click conversion increases as the mPEGMA mole fraction in the polymer brush increases, presumably because the brush becomes more hydrophilic. In addition, the IR absorbances associated with the added PEG chain (C-O stretching bands at 1100 and 1350, and C-H stretching at 2900 cm^{-1}) and increases in film thickness (Figure 9) are consistent with higher reaction yields for copolymers with more mPEGMA. The most hydrophilic brush, poly(AzPMA-*co*-mPEGMA) (AzPMA/mPEGMA = 25/75) nearly doubled in thickness, from 47 nm to 92 nm after the click reaction, whereas the thickness of the poly(AzPMA) brush changed by $<10\%$ under identical reaction conditions (Figure 9). Azide consumption tracked the increase in film thickness; $>60\%$ for AzPMA/mPEGMA = 25/75 and $<15\%$ for poly(AzPMA). The expansion of the poly(AzPMA-*co*-mPEGMA) (25/75) film and the IR analyses confirm that the click reaction occurs in the bulk of the film, while poly(AzPMA) films likely react primarily on their surface.

In a study of binding amino-terminated PEG to polymer brushes with activated esters in DMF, Schuh and Rhe found that binding efficiency of amino-terminated PEGs decreased with increased molecular weight.⁶⁷ The conversion for PEG 5000 was $\sim 50\%$, and decreased to less than 20% for PEG 22,500. Their key conclusion was that chain entropy prevents high molecular weight PEG chains from penetrating the brush, and therefore, binding events occur at the outer periphery of the brush. Our experiments used PEG 5000, which falls in the range where the chain entropy is moderate. It would be interesting to replicate some aspects of Schuh and Rhe's work in poly(AzPMA-*co*-mPEGMA) brushes, such as the binding efficiency as a function of PEG molecular weight, and brush thickness, especially in the context of binding biomolecules in aqueous media. Also, since the AzPMA unit is hydrophobic, binding efficiency may increase when the AzPMA content is <25 mol % in poly(AzPMA-*co*-mPEGMA) brushes.

Conclusions

Azidopropyl methacrylate (AzPMA) readily polymerizes from surfaces using a CuCl/PMDETA catalyst and provides predictable polymer film thicknesses, attaining 200 nm polymer films in ~4 hours at 50 °C. IR analyses of copolymerizations of AzPMA with either EGMA or mPEGMA show that the monomers enter into the polymer brush randomly. Since poly(AzPMA) is hydrophobic, efficient click reactions require solvents that swell the polymer brush, and dissolve the Cu catalyst and the alkynylated substrate. Using DMF as the solvent, the reaction of an alkynylated dye was 60% complete in one minute at 50 °C, irrespective of the copolymer composition. In contrast, aqueous click reactions require a hydrophilic polymer brush, and the yield from an alkynylated mPEG ($M_n \sim 5000$) depended on the composition of the polymer brush. The most hydrophilic copolymers, i.e. those with the highest fraction of mPEGMA, immobilized the most mPEG, and gave the highest increases in film thickness. Aqueous click reactions offer an alternative to ester or amide formation for the covalent immobilization of biomacromolecules in brushes, but achieving effective reactions requires control over the brush hydrophilicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to the U.S. National Institutes of Health (GM080511) for funding this work.

REFERENCES

- (1). Golas PL, Matyjaszewski K. QSAR Comb. Sci. 2007; 26:1116.
- (2). Braunecker WA, Matyjaszewski K. Prog. Polym. Sci. 2007; 32:93.
- (3). Barbey R, Lavanant L, Paripovic D, Schuwer N, Sugnaux C, Tugulu S, Klok HA. Chemical Reviews. 2009; 109:5437. [PubMed: 19845393]
- (4). Matyjaszewski K, Tsarevsky NV. Nat. Chem. 2009; 1:276. [PubMed: 21378870]
- (5). Grubbs RB. Polym. Rev. 2011; 51:104.
- (6). Bruening ML, Dotzauer DM, Jain P, Ouyang L, Baker GL. Langmuir. 2008; 24:7663. [PubMed: 18507420]
- (7). Grajales ST, Dong XJ, Zheng Y, Baker GL, Bruening ML. Chem. Mat. 2010; 22:4026.
- (8). Lee BS, Lee JK, Kim WJ, Jung YH, Sim SJ, Lee J, Choi IS. Biomacromolecules. 2007; 8:744. [PubMed: 17291100]
- (9). Jain P, Vyas MK, Geiger JH, Baker GL, Bruening ML. Biomacromolecules. 2010; 11:1019. [PubMed: 20187657]
- (10). Sanjuan S, Tran Y. Macromolecules. 2008; 41:8721.
- (11). Rhe, J.; Ballauff, M.; Biesalski, M.; Dziezok, P.; Grohn, F.; Johannsmann, D.; Houbenov, N.; Hugenberg, N.; Konradi, R.; Minko, S.; Motornov, M.; Netz, RR.; Schmidt, M.; Seidel, C.; Stamm, M.; Stephan, T.; Usov, D.; Zhang, HN. Polyelectrolytes with Defined Molecular Architecture I. Vol. Vol. 165. Springer-Verlag; Berlin: Berlin: 2004. p. 79
- (12). Minko, S., editor. Responsive Polymer Materials: Design and Applications. Blackwell Publishing; Boston: 2006.
- (13). Advincula, RCB.; J., W.; Caster, KC.; Rhe, J., editors. Polymer Brushes. Wiley-VCH Verlag; Weinheim: 2004.
- (14). Sumerlin BS, Vogt AP. Macromolecules. 2010; 43:1.
- (15). Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. Angew. Chem.-Int. Edit. 2002; 41:2596.
- (16). Kolb HC, Finn MG, Sharpless KB. Angew. Chem.-Int. Edit. 2001; 40:2004.
- (17). Hoyle CE, Lowe AB, Bowman CN. Chem. Soc. Rev. 2010; 39:1355. [PubMed: 20309491]

- (18). Binder WH, Sachsenhofer R. *Macromol. Rapid Commun.* 2007; 28:15.
- (19). Lutz JF. *Angew. Chem.-Int. Edit.* 2007; 46:1018.
- (20). Nandivada H, Jiang XW, Lahann J. *Adv. Mater.* 2007; 19:2197.
- (21). Binder WH, Sachsenhofer R. *Macromol. Rapid Commun.* 2008; 29:952.
- (22). Johnson JA, Finn MG, Koberstein JT, Turro NJ. *Macromol. Rapid Commun.* 2008; 29:1052.
- (23). Lundberg P, Hawker CJ, Hult A, Malkoch M. *Macromol. Rapid Commun.* 2008; 29:998.
- (24). Meldal M. *Macromol. Rapid Commun.* 2008; 29:1016.
- (25). van Dijk M, Rijkers DTS, Liskamp RMJ, van Nostrum CF, Hennink WE. *Bioconjugate Chem.* 2009; 20:2001.
- (26). Chu CH, Liu RH. *Chem. Soc. Rev.* 2011; 40:2177. [PubMed: 21212875]
- (27). Fu R, Fu GD. *Polym. Chem.* 2011; 2:465.
- (28). Sumerlin BS, Tsarevsky NV, Louche G, Lee RY, Matyjaszewski K. *Macromolecules.* 2005; 38:7540.
- (29). Van Camp W, Germonpre V, Mespouille L, Dubois P, Goethals EJ, Du Prez FE. *React. Funct. Polym.* 2007; 67:1168.
- (30). Zhang Y, He H, Gao C, Wu JY. *Langmuir.* 2009; 25:5814. [PubMed: 19374339]
- (31). Li Y, Yang JW, Benicewicz BC. *J. Polym. Sci. Pol. Chem.* 2007; 45:4300.
- (32). Yuan YY, Du Q, Wang YC, Wang J. *Macromolecules.* 2010; 43:1739.
- (33). Gao HF, Matyjaszewski K. *J. Am. Chem. Soc.* 2007; 129:6633. [PubMed: 17465551]
- (34). Tsarevsky NV, Bencherif SA, Matyjaszewski K. *Macromolecules.* 2007; 40:4439.
- (35). Soto-Cantu E, Lokitz BS, Hinestrosa JP, Deodhar C, Messman JM, Ankner JF, Kilbey SM. *Langmuir.* 2011; 27:5986. [PubMed: 21506527]
- (36). Xu LQ, Wan D, Gong HF, Neoh KG, Kang ET, Fu GD. *Langmuir.* 2010; 26:15376. [PubMed: 20839788]
- (37). Wu T, Ge ZS, Liu SY. *Chemistry of Materials.* 2011; 23:2370.
- (38). Song WT, Xiao CS, Cui LG, Tang ZH, Zhuang XL, Chen XS. *Colloids and Surfaces B-Biointerfaces.* 2012; 93:188.
- (39). Cai T, Wang R, Yang WJ, Lu SJ, Neoh KG, Kang ET. *Soft Matter.* 2011; 7:11133.
- (40). Rahane SB, Hensarling RM, Sparks BJ, Stafford CM, Patton DL. *Journal of Materials Chemistry.* 2012; 22:932.
- (41). Hensarling RM, Doughty VA, Chan JW, Patton DL. *Journal of the American Chemical Society.* 2009; 131:14673. [PubMed: 19778016]
- (42). Fleischmann S, Hinrichs K, Oertel U, Reichelt S, Eichhorn KJ, Voit B. *Macromolecular Rapid Communications.* 2008; 29:1177.
- (43). Gao HF, Louche G, Sumerlin BS, Jahed N, Golas P, Matyjaszewski K. *Macromolecules.* 2005; 38:8979.
- (44). Crownover E, Duvall CL, Convertine A, Hoffman AS, Stayton PS. *J. Controlled Release.* 2011; 155:167.
- (45). Ricardo CL, Pintauer T. *Eur. J. Inorg. Chem.* 2011:1292.
- (46). He H, Zhang Y, Gao C, Wu J. *Chem. Commun. (Cambridge, U. K.).* 2009:1655.
- (47). Zhang Y, He H, Gao C, Wu J. *Langmuir.* 2009; 25:5814. [PubMed: 19374339]
- (48). Talelli M, Morita K, Rijcken CJF, Aben RWM, Lammers T, Scheeren HW, van NCF, Storm G, Hennink WE. *Bioconjugate Chem.* 2011; 22:2519.
- (49). Canalle LA, van BSS, de HLT, van HJCM. *Adv. Funct. Mater.* 2009; 19:3464.
- (50). Canalle LA, van d. K. M. Overhand M, van HJCM. *Macromol. Rapid Commun.* 2011; 32:203. [PubMed: 21433141]
- (51). Li Y, Benicewicz BC. *Macromolecules.* 2008; 41:7986.
- (52). Mespouille L, Coulembier O, Paneva D, Degee P, Rashkov I, Dubois P. *J. Polym. Sci., Part A: Polym. Chem.* 2008; 46:4997.
- (53). Gehan H, Fillaud L, Felidj N, Aubard J, Lang P, Chehimi MM, Mangeney C. *Langmuir.* 2010; 26:3975. [PubMed: 20039598]

- (54). Quemener D, Le Hellaye M, Bissett C, Davis TP, Barner-Kowollik C, Stenzel MH. *J. Polym. Sci. Pol. Chem.* 2008; 46:155.
- (55). Wiltshire JT, Qiao GG. *J. Polym. Sci. Pol. Chem.* 2009; 47:1485.
- (56). Sanjuan S, Tran Y. *J. Polym. Sci. Pol. Chem.* 2008; 46:4305.
- (57). Daugaard AE, Hvilsted S, Hansen TS, Larsen NB. *Macromolecules.* 2008; 41:4321.
- (58). Schoffelen S, Lambermon MHL, van Eldijk MB, van Hest JCM. *Bioconjugate Chem.* 2008; 19:1127.
- (59). Snaith HJ, Whiting GL, Sun BQ, Greenham NC, Huck WTS, Friend RH. *Nano Lett.* 2005; 5:1653. [PubMed: 16159201]
- (60). Whiting GL, Snaith HJ, Khodabakhsh S, Andreasen JW, Breiby D, Nielsen MM, Greenham NC, Friend PH, Huck WTS. *Nano Lett.* 2006; 6:573. [PubMed: 16522065]
- (61). Bao ZY, Bruening ML, Baker GL. *J. Am. Chem. Soc.* 2006; 128:9056. [PubMed: 16834378]
- (62). Zhang JY, Zhou YM, Zhu ZY, Ge ZS, Liu SY. *Macromolecules.* 2008; 41:1444.
- (63). Pintauer T, Matyjaszewski K. *Coord. Chem. Rev.* 2005; 249:1155.
- (64). Tsarevsky NV, Matyjaszewski K. *J. Polym. Sci. Pol. Chem.* 2006; 44:5098.
- (65). Chan TR, Hilgraf R, Sharpless KB, Fokin VV. *Org. Lett.* 2004; 6:2853. [PubMed: 15330631]
- (66). Golas PL, Tsarevsky NV, Sumerlin BS, Matyjaszewski K. *Macromolecules.* 2006; 39:6451.
- (67). Schuh C, Rhe J. *Macromolecules.* 2011; 44:3502.

Figure 1.

Evolution of the ellipsometric brush thickness with time for AzPMA polymerization from initiator monolayers on Au using the following catalysts: CuBr/Me₄Cyclam/dnNbpy)/DMF at 50 °C (x), CuCl/PMDETA/DMF at 50 °C (■), CuCl/PMDETA/CuBr₂/DMF at 50 °C (▲), CuCl/bpy/CuBr₂/isopropanol at 50 °C (◆) and CuCl/bpy/CuBr₂/isopropanol at RT (●). Each point represents a different film.

Figure 2.

Evolution of the ellipsometric brush thickness with time during poly(AzPMA), poly(AzPMA-*co*-mEGMA), and poly(mEGMA) growth from initiator monolayers on gold. The polymerization occurred with several monomer ratios using CuCl/PMDETA/DMF as the catalyst at 50 °C. AzPMA/mEGMA (100/0), AzPMA/mEGMA (75/25), AzPMA/mEGMA (50/50), AzPMA/mEGMA (25/75), × AzPMA/mEGMA (0/100). Each point represents a different film.

Figure 3.

Reflectance FTIR spectra of poly(AzPMA), poly(AzPMA-*co*-mEGMA), and poly(mEGMA) brushes grown from initiators on gold using different comonomer ratios. (a) AzPMA/mEGMA (0/100) (b) AzPMA/mEGMA (25/75) (c) AzPMA/mEGMA (50/50) (d) AzPMA/mEGMA (75/25) (e) AzPMA/mEGMA (100/0).

Figure 4.

Composition of poly(AzPMA-*co*-mEGMA) brushes on gold after 5 minutes of polymerization, as a function of the mole fraction in the polymerization solution. To determine the mole fraction of AzPMA in each copolymer, we assumed that the ratio of the areas of the azide and carbonyl bands are proportional to the fraction of AzPMA in the copolymer. The pure poly(AzPMA) served as a single point calibration. The thickness of each film was ~50 nm.

Figure 5.

Composition of poly(AzPMA-*co*-mPEGMA) brushes on gold after 5 minutes of polymerization, as a function of the comonomer ratio in the polymerization solution. We calculated the mole fraction of AzPMA in each copolymer from the ratio of the areas of azide and carbonyl bands.

Figure 6. UV-vis spectra of ITO-supported ~180 nm-thick poly(AzPMA-*co*-mPEGMA) (50/50) brushes after reaction with alkynyl-modified fluorescein in DMF for various times. Blue line: 5 min, red line: 1 h and green line: 4 h.

Figure 7.

Absorbance at $\lambda_{\max}$ (463 nm) and dye molecule binding as a function of the percentage of AzPMA in 200 ± 30 nm poly(AzPMA-*co*-mPEGMA) brushes on ITO after a 5 minute click reaction. The dye molecule binding was determined from the absorbance at $\lambda_{\max}$ assuming an extinction coefficient of $52200 \text{ cm}^2/\text{mol}$.

Figure 8.

Representative fluorescence microscopy images of ITO coated with ~180 nm of poly(AzPMA-*co*-mEGMA) (1:1) and reacted for 5 min with alkynylated fluorescein (left), and a control ITO surface (right), treated identically but without the copper catalyst. The images were recorded using equal lighting and camera settings.

Figure 9.

Increases in thicknesses and percent disappearance of azide infrared absorbances ($\sim 2100\text{ cm}^{-1}$) after click reactions of various poly(AzPMA-*co*-mPEGMA) brushes with alkynylated mPEG 5000 for 12 hours at room temperature. The initial brushes were $\sim 50\text{ nm}$ thick.