HIGH DENSITY SCAFFOLDING OF FUNCTIONAL POLYMER BRUSHES: A FUNDAMENTAL STUDY TOWARDS THE DEVELOPMENT OF A UNIVERSAL POLYMER THIN FILM TEMPLATE

by

SARA VICTORIA ORSKI

(Under the Direction of Jason Locklin)

ABSTRACT

The fabrication of tunable polymer coatings is of interest to materials science and biotechnology for the development of microarray chips and other small scale surface devices. Polymeric activated esters have been demonstrated as a facile route to make molecular and biomolecular polymer conjugates. This dissertation details the design, synthesis, and applications of poly(n-hydroxysuccinimide 4-vinyl benzoate) (poly (NHS4VB)) brushes as a versatile method for creating scaffolds for post-polymerization modification with spatial and temporal control of surface functionality.

The active ester brushes were designed to have uniform morphology, controlled polymerization rate, and block copolymer–tuned microenvironments using living polymerization methods. Poly(NHS4VB) brushes were derivatized with various reactive alkynes that differ in relative rates of activity in azide-alkyne cycloaddition reactions. Relative rates of polymer brush functionalization to their solution counterparts are compared to help to determine the extent of polymer brush functionalization and elucidate the influence of polymer brush architecture for post-polymerization functionalization reactions.

Poly(NHS4VB) brushes can also template multicomponent surfaces with spatially resolved functionality by both "top down" and "bottom up" methods. Poly(NHS4VB) brushes modified with a cyclopropenone-protected dibenzocyclooctyne were patterned using photolithography to selectively activate a strained alkyne moiety for catalyst-free cycloaddition with azides. Bottom up templating of multifunctional surfaces was demonstrated by orthogonal self-assembly of catechol functionalized initiators and subsequent active ester polymerization on titanium oxide selectively in the presence of silicon oxide. Further incorporation of chemical and spatial complexity was introduced by synthesis of a second functional polymer brush, poly(ethynyl styrene), from the remaining silicon oxide sites. High fidelity patterns of surface functionality were demonstrated in both top down and bottom up patterning methods by fluorescence microscopy of two fluorescent dyes selectively bound to the brush surfaces. Selective immobilization of dual-functional polymer brushes can be pragmatic to the design of customizable polymer brush coated nanostructures for tunable nanotechnology applications. This dissertation is the culmination of the design, fabrication, and characterization of easily patterned and customizable polymer brush scaffolds for small scale devices and nanomaterial uses.

INDEX WORDS: polymer brushes, active esters, post-polymerization modification, orthogonal self-assembly, copper-free click chemistry

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DEDICATION

To my husband, Zachary: Thank you for being the rock that keeps me grounded, the partner that walks beside me, and the best friend that I can "geek out" with at the dinner table. All of your love and support during graduate school has been invaluable to me. The last few years have been jam-packed with adventures for us: adopting our dog Tiki, getting married, you getting your doctorate and me following right behind you. I am excited to tackle new adventures in our life together, and maybe fill a photo album or two along the way. I love you.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Polymeric Thin Films

The development of small-scale sensors and devices requires particular focus on the interface between the bulk device and the surrounding environment. Polymeric thin films have traditionally been used as protective coatings and paints. Specialized polymer thin films, where the properties of the polymer can tailor surface properties of the bulk material, have been recently employed to develop new materials for microelectronic and biomedical devices.¹⁻⁷ Polymers can be fabricated to tune interfacial properties such as adhesion, friction, wetting, and adsorption of molecules from the environment.⁸⁻¹⁰ Surface properties of polymeric thin films are dictated not only by the chemistry incorporated into the polymer chains, but also by the method of attachment to a surface.

Polymeric thin films can interact with a surface in two ways, through physical deposition of a thin film (physisorption), or by covalent attachment to the surface (chemisorption). Surface modification by physisorption is governed by weak intermolecular forces between the polymer thin film and the substrate. Deposition methods such as painting, spray-coating, spin-coating, dip-coating or doctor blading can deposit well controlled, homogenous polymer thin films with virtually no upper limit to film thicknesses.¹¹ The weak forces between polymer and substrate can lead to

destruction of the thin film through four methods: desorption, displacement, delamination, and dewetting. Polymer thin films prepared under non-ideal conditions, or not under ultra-high vacuum, are prone to desorption and displacement due to competitive interactions with water, ions, or polyelectrolytes. Mechanical stress at the surface causes delamination, often induced by large temperature changes or swelling of the polymer in the surrounding system.¹¹ Dewetting occurs when there is a mismatch of surface tension (γ) between the substrate and polymer coating ($\gamma_{substrate} < \gamma_{polymer}$) and the polymer film is heated above its glass transition temperature (Tg).¹¹ These limitations of physisorption techniques have led to the use of chemisorption as an alternative to physical deposition methods.

Covalent attachment of a polymer thin film to the surface provides enhanced stability to polymer surface modification over physical adsorption methods. The first examples of covalent surface modification were demonstrated with the formation of self-assembled monolayers (SAM)s.¹² Self-assembled monolayers are organic assemblies at the surface which consist of a functional "head group" that will react with a complimentary functional group on the surface, a main chain organized by van der Waals interaction, and a terminal group, whose functionality defines surface chemistry.¹³ SAMs have been used extensively to modify and tune surface chemistry with semiconductors, metals, and metal oxides. ^{14,15} They can introduce virtually any functional group onto a surface, but have some limitations. SAMs, which are only on the order of a few nanometers thickness, are fragile and often contain defects across the surface. Functionalization density of a surface with SAMs is limited by the two-dimensional surface area of the substrate. Polymer "brushes" are a monolayer of

macromolecules, where covalently-bound polymer chain ends expose more functional groups along the 3D polymer backbone. A depiction SAMs and polymer brushes can be seen in Figure 1.1.



Figure 1.1. Cartoon depiction of SAMs (left) and polymer brushes (right). The red circles represent functional groups covalently bound to the surface.

Polymer Brushes

Polymer brushes are polymer chain ends that are covalently tethered to a surface with a density high enough to alter the unperturbed solution dimensions of the chains.¹⁶ Films generated from polymer chains in extended conformations exhibit surface phenomena that are different than polymers deposited onto a substrate from solution, such as wetting, phase-segregation, adsorption of molecules and macromolecules, lubrication, and diffusion control.¹⁷ These unique properties of polymer brushes over other thin polymer films and microgels are dictated by the ordered, extended chains that are perpendicular to the surface. The ordered chains are extended on the densely grafted surface to balance two opposing energies: the entropic energy gained by a random walk configuration of the polymer chains and the energetic favorability of the chains to be highly solvated and non-overlapping.¹⁸ This energy balance determines many physical

properties of polymer brushes such as lower compressibility or high stiffness not observed in other polymer thin films. In addition, the extended conformation yields a greater surface area to the polymer thin film. The three dimensional brush allows for greater numbers of functional groups per unit area, allowing the side chain functionality to have greater influence on the interfacial chemistry.¹⁷ In contrast, thin polymer films and microgels are coiled and intercalated at the surface, which causes poor solvation of the overlapping chains and buried functional groups within the film layers. Several recent reviews have been dedicated to topics including the synthesis and applications of polymer brushes in fields like biomaterials, sensors, and nanotechnology.^{17,19-21}

The degree to which polymer chains in the brush regime interact with each other to exhibit these surface phenomena is largely determined by their method of formation. Polymer brushes can be formed in a "grafting to" approach by synthesizing a polymer in solution with a reactive functional group at one end of the chain, which is covalently attached to a complimentary functional group on the surface.^{22,23} This process, however, is diffusion limited, which keeps polymer brush grafting density low and limits the degree of chain extension from the surface. As polymer chains are more likely to be in their entropically favored random coil configuration in solution, they remain somewhat coiled once attached to the surface, forming a blocking layer to surrounding reaction sites. These non-interacting random coils are referred to as the "mushroom regime," which in good solvents have a thickness (h) proportional to their degree of polymerization (N). A depiction of grafting to and grafting from polymer brushes is shown in Figure 1.2.



Figure 1.2. Polymer brushes from a "grafting to" and "grafting from" approach.

Polymer brushes are also synthesized by the direct polymerization from an immobilized initiator species on the surface in a "grafting from" approach.^{18,24} Chains that are grown through surface initiated polymerization (SIP) have higher grafting density, σ , which is typically reported in units of chains/nm². Figure 1.3 illustrates the different polymer brush regimes of increasing grafting density.



Figure 1.3. Depiction of mushroom, crossover, and brush regimes of grafted polymer thin films in relation to brush thickness (h) and reduced tethering density (Σ). The figure is not drawn to scale.

The distance between grafted polymer chains is typically much less than the size of the polymer coil, or Flory radius.²⁵ The ratio of grafted chains that occupy the same surface area as a free overlapping chain is known as the reduced tethering density, represented by Σ in Figure 1.3. This value is an indication of how stretched the polymer chains are. Reduced tethering density can be calculated by Equation 1.1,

$$\Sigma = \sigma \pi R_g^2 \qquad (\text{Equation 1.1})$$

$$\sigma = \frac{h\rho N_A}{M_n}$$
 (Equation 1.2)

where R_g is the radius of gyration of the grafted polymer chains. Equation 1.2 defines grafting density in relation to brush thickness (h), polymer bulk density (ρ), Avogadro's number (N_A), and the number average molecular weight of the polymer (M_n).

This highly extended polymer chain configuration is known as the "brush regime," where the brush thickness is proportional to $N\sigma^{1/3}$.²⁴ Due to excluded volume effects, the polymer chain will forcibly grow in a more extended conformation as the polymerization proceeds. While this extended conformation has entropic energy loss, the stretched chains can interact more collectively. In this geometry, selective control of the polymer brush nano-environment, brought about by conformational changes using stimuli such as temperature or polymer solvation, can dramatically alter film properties such as thickness and morphology. These properties are vital, and can be used to generate and control polymer brush interfaces.

Living Polymerizations

Bimolecular termination and chain transfer of radical polymerization limit the lifetime of propagating radicals in conventional free radical polymerization.²⁶ In free radical polymerizations, as radicals are generated, molecular weight builds quickly with reaction time, but plateaus as available monomer concentration decreases and chain termination events prevent further polymerization. Living radical polymerization has been achieved to minimize bimolecular termination and prolong the lifetime of the living radicals on the order of hours through the introduction of a dormant state for the propagating species. The prolonged lifetime is achieved by the reversible transfer or termination of the propagating radical controlled by a mediating agent.²⁶ This reversible activation and deactivation allows molecular weight to build slowly, affording low

polydispersities in the chains and controlling polymer molecular weight with reaction time. Figure 1.4 illustrates the general differences in how molecular weight builds in living versus conventional free radical polymerizations.



Figure 1.4. General depiction of how molecular weight builds with time in free radical and living radical polymerization mechanisms.

Living polymerizations initiated from the surface can aid in the fabrication of homogenous polymer thin films. Conventional free radical polymerization from the surface can have a high polydispersity and poor control of chain length. Longer chains on the outer fringe of the polymer brush can mask the densely packed chains below.²⁷ Polymer brushes that are generated through living or controlled polymerization techniques are especially attractive towards the fabrication of micro- and nano-structured surfaces due to the precise control over molecular weight and low polydispersity.¹⁷ These techniques include, but are not limited to, atom transfer radical polymerization (ATRP),

nitroxide mediated polymerization (NMP), and ring opening metathesis polymerization (ROMP) reactions.^{21,28-36} Use of living polymerizations adds a further degree of control over surface morphology as the extended polymer chains will have a very small molecular weight distribution, making a more homogenous collective polymer response. Low polydispersities obtained with living radical polymerization yield well-defined high-density polymer brushes as has been previously discussed in the literature. ²⁷

Atom Transfer Radical Polymerization

One specific polymerization mechanism of living radical polymerization is known as atom transfer radical polymerization. ATRP is mediated by a transition metal-ligand complex which undergoes a reversible redox process,³⁷ as shown in Figure 1.5.



Figure 1.5. General reaction scheme of atom transfer radical polymerization (ATRP).

The control of propagation of ATRP arises from the equilibrium between activation of the propagating radical by halogen transfer from the alkyl halide to the metal-ligand complex (k_{act}) and deactivation back to the dormant state by the reduction of the metal ligand complex and subsequent reformation of the alkyl halide initiator (k_{deact}). For ATRP to be a controlled reaction, k_{deact} must be significantly greater than k_{act} .²⁶ The propagating radical, R[•], can then either react with an unsaturated monomer M_o with a propagation rate of k_p , or undergo a permanent termination reaction with a rate of k_t . The cycle of activation, propagation, and deactivation continues until the all monomer is consumed or the reaction is quenched by exposure to oxygen. The alkyl halide initiator remains on the polymer chain end after the reaction is quenched, making the ATRP polymer a macroinitiator for further polymerization with a different monomer to form block copolymers.

The rate of ATRP is determined by the k_{act}/k_{deact} equilibrium, which is controlled by various reaction parameters, including choice of transition metal, ligand, solvent, heat, and optional addition of sacrificial initiator³⁸ or copper (II) deactivator^{39,40} (to increase k_{deact} and shift the equilibrium to the dormant state). The metal catalyst most often used in ATRP is a copper (I) halide. The ligands used are commercially available multidentate amine ligands, which have been studied extensively to control the equilibrium constant for ATRP.⁴¹ The role of the ligand is to help solubilize the copper in the organic reaction system and will coordinate well with the copper center in both the +1 and +2 oxidation states. ATRP is compatible with protic and aprotic organic solvents, and even water, with noted rate acceleration in polar solvents.^{42,43} ATRP is the most versatile of the living radical polymerization methods both in solution and from the surface.³⁶

The delicate redox equilibrium makes ATRP sensitive to oxygen, so materials must be degassed prior to reaction. Newer adaptations on ATRP, however, allow for the toleration of some oxygen present, including activators regenerated by electron transfer (ARGET),^{44,45} initiators for continuous activator regeneration (ICAR),⁴⁶ and electrochemically mediated ATRP (eATRP),⁴⁷ which all regenerate copper (I) *in situ*.

Nitroxide Mediated Polymerization

Stabilized free radical polymerization has also been established using a radical deactivator to mediate the propagating radical concentration. Nitroxide mediated polymerization (NMP) is initiated by an alkoxyamine (Figure 1.6) in which C-O bond decomposes at high temperatures to form a reactive radical and 2, 2, 6, 6-tetramethyl-1-piperidinoxyl (TEMPO). Initiation has also been achieved using a mixture of conventional free radical initiator, such as azobisisobutyronitrile (AIBN), and a nitroxide radical. ^{48,49}



Figure 1.6. General scheme of nitroxide mediated polymerization of styrene with an alkoxyamine initiator. The C-O bond highlighted in red is where the radicals are thermally generated from cleavage of the C-O bond.

The nitroxide radical most often used is TEMPO, which is stabilized due to steric hindrance and cannot self-terminate. TEMPO radicals will deactivate propagating chains to control the steady state concentration of radicals during the reaction, analogously to ATRP. NMP affords the lowest polydispersity of reaction with styrene and styrene derivatives and is less compatible with acrylates and methacrylates, common to ATRP.⁵⁰ Thermal activation of radicals is not as immediate as with ATRP and requires longer reaction times.²⁶ Homopolymer and block copolymer brushes with controlled molecular weights have been synthesized by surface initiated NMP.^{51,52}

Ring Opening Methathesis Polymerization

Strained cyclic olefins have been polymerized using ring opening metathesis polymerization (ROMP). No radical species is formed in this method, but polymerization is driven by the relief of ring strain a metal carbene complex, which ring opens the cyclic olefin, relieving ring strain. A number of metal-carbene catalysts have been synthesized based on molybdenum,⁵³ tungsten⁵⁴ and ruthenium.^{55,56} Ruthenium–carbene catalysts, known as Grubbs catalysts, are the most tolerant of moisture, air, and other functional groups.^{26,55} A depiction of ROMP using a Grubbs catalyst is shown in Figure 1.7. The Grubbs catalyst coordinates with the alkyne in the cyclic olefin to initiate the reaction, followed by cleavage of the alkene π -bond, forming a 4-membered metallobutane cyclic intermediate.⁵⁷ Rearrangement forms a double bond and a metal-carbene propagation center for further olefin metathesis. Common monomers for ROMP include cyclic and bicyclic compounds such as norbornene, cyclooctatetrene, cyclooctadiene derivatives. ROMP is a living polymerization that gives low polydispersity homopolymers and block copolymers at low temperature reaction conditions, from 25°C to 50°C.²⁶ These mild

reaction conditions have led to several examples describing surface initiated ROMP from alkene or cyclic olefin monolayers.⁵⁸⁻⁶¹



Figure 1.7. General scheme representing ring opening metathesis polymerization (ROMP).

General Description of Post-Polymerization Modification

In the past, designing specialized functionality along the backbones of polymer chains had required one of two strategies: (1) the synthesis of a monomer bearing a desired functionality, followed by experimentation and optimization of polymerization conditions for that monomer that afford the desired molecular weight and polydispersity and (2) modification of polymer chains pot-polymerization by derivatization of a common functional group (such as an alcohol) on the pendant group extending from the aliphatic polymer backbone. This stepwise derivatization of polymer chains involved several permutations of the polymer to get the desired functionality and were often not quantitative and prone to side reactions through several transformations.^{62,63}

Gauthier describes direct polymerization of a monomer bearing "chemoselective post-polymerization modification.⁶⁴ handles" as method for quantitative а Polymerization of a functional monomer designed for post-polymerization derivatization must be inert to the polymerization conditions and quantitatively convert with a complimentary functional group in solution to attach desired functionalities.⁶⁴ The development of living polymerizations such as ATRP, ROMP, NMP, and reversible addition fragmentation chain transfer polymerization (RAFT) are more tolerant of different functional groups than their predecessor polymerization mechanisms such as anionic and cationic polymerization. However, functional group limitations that still exist for living radical polymerizations, such as the incompatibility of carboxylic acid monomers with ATRP, can be overcome by the polymerization of functional monomers and post-polymerization modification. Post-polymerization modification of functional polymers is a straightforward way to overcome functional group incompatibilities in polymerization, and to quickly introduce new types of moieties onto the repeat units of polymer chains, increasing the available polymer conjugation methods for combinatorial Figure 1.8 depicts the synthesis and post-polymerization materials discovery. modification of derivatizable polymer chains.



Figure 1.8. Polymerization and derivatization of a polymer chain containing a functionalizable pendant group.⁶⁵ Reprinted with permission from Wiley Interscience 2008.

Several classes of polymers amenable to single step post-polymerization modification exist, the most common of which is the modification polymers containing activated ester functional groups. Activated esters contain an excellent leaving group, prompting the rapid and quantitative functionalization with amine nucleophiles under mild conditions. Alcohols can also be added to active esters, but require harsher reaction conditions such as strong base and heat.⁶⁶ Several polymeric active esters such as n-hydroxysuccinimide,⁶⁶⁻⁷⁴ pentafluorophenyl,⁷⁵⁻⁷⁸ p-nitrophenyl,⁷⁹⁻⁸² and dicarboxyimide⁸³ functionalized esters have been synthesized. Polymers and polymer brushes bearing an azlactone group can also be quantitatively functionalized in aqueous environments

without competitive hydrolysis.⁸⁴⁻⁸⁶ Several other functional polymers besides active esters have been synthesized for post-polymerization modification such as epoxides,⁸⁷⁻⁹³ activated alkenes,⁹⁴⁻⁹⁶ thiols,^{97,98} pyridyldisulfides,^{72,99-101} alkyne/azide cycloaddition,^{102-¹¹² and aldehydes and ketones.¹¹³⁻¹¹⁷}

N-Hydroxysuccinimide Activated Esters

N-hydroxysuccinimide (NHS) esters are the most common reactive group of activated esters and are a widely used post-polymerization modification method to conjugate biological analytes to solid supports in their native form, where the n-terminus of peptides, or lysine side chains covalently bind at the active ester site.¹¹⁸ While NHS esters are hydrolytically stable in aqueous systems,⁷⁴ their rate of hydrolysis increases in basic environments.¹¹⁸⁻¹²⁰ Also, side reactions involving ring opening or glutarimide formation between two active esters are possible.¹²¹ Functionalization strategies can minimize side reactions in active ester functionalization, such as using a high concentration of the desired amine moiety to be attached or functionalizing in organic solvents in the presence of a proton acceptor such as triethylamine or 4-dimethylamino)pyridine.^{69,118}

More recently, the synthesis of polymers containing active ester groups has emerged as a popular strategy for coupling synthetic and biomacromolecules.^{65,84,122-128} Pendant group functionalization is straightforward and shows first order reaction kinetics with very fast reaction times.¹²⁹ It has been shown that synthetic polymers coupled to proteins can improve the overall protein stability, solubility, and biocompatibility, and are being utilized in the fields of biotechnology and medicinal research.^{130,131} Controlled polymerizations containing active esters, including the development of block copolymers, have been achieved in solution by controlled polymerization techniques such as ATRP,¹³²⁻¹³⁴ NMP,¹³⁵ ROMP,^{78,136-138} and reversible addition–fragmentation chain transfer (RAFT) polymerization.¹³⁹⁻¹⁴²

As controlled polymer architectures of active ester polymers have advanced in solution, there are few reports of these polymers grafted from solid interfaces. The Rühe group has polymerized *n*-methacryloyl-β-alanine succinimide ester via a free-radical polymerization from a surface bound AIBN initiator and demonstrated the functionalization of the activated ester with small molecules and oligomers on polymer thicknesses up to 80 nm.¹⁴³ Cullen and co-workers used ATRP to grow polymer brushes of 2-vinyl-4,4-dimethyl azlactone from a surface to immobilize RNAse A and showed the enzyme maintained activity while covalently attached to the polymer matrix.⁸⁵

Post-Polymerization Modification by Click Chemistry

There is a recent interest in the incorporation of Sharpless-type "click" chemistry into post-polymerization modification of polymers in solution¹⁰⁵⁻¹¹⁰ and grafted on the surface.^{144,145} The alkyne-azide Huisgen 1, 3-dipolar cycloaddition is emerging as an ideal coupling approach, as it is highly selective and tolerant of other functional groups. The straightforward cycloaddition reaction is compatible with protic, aprotic, and aqueous solvent conditions, has a rapid rate, and proceeds with high quantitative yields ^{107,146-148} Click chemistry reactions are especially appealing for biological attachment due to their facile incorporation into biomolecules through post-synthetic modification^{149,150} enzymatic transfer,^{151,152} and through azide-modified nutrients for metabolic functionalization.¹⁵³ While conventional copper (I)-catalyzed alkyne azide cycloaddition (CuAAC) is ideal for many applications,¹⁵⁴⁻¹⁵⁶ the cytotoxicity of the Cu catalyst can
limit bioorthogonal conjugation. Bertozzi and co-workers designed the first alternative to using CuAAC for alkyne/azide cycloaddition by synthesizing a difluoronated cyclooctyne (DIFO) derivative.^{157,158} They fluorescently labelled the azide-functionalized glycans in live zebra fish using the DIFO without metal catalysis.¹⁵⁸

Recently, catalyst-free click reactions have emerged that utilize a reactive high energy, "strained" cycloalkyne to promote the [3+2] cycloaddition with comparable reaction rates while avoiding toxic metal catalysts.^{157,159-162} Previous literature has described catalyst free cycloaddition as "strain-promoted" by the cyclooctyne, although there is not a significant amount of energy released upon relieving the ring strain.¹⁶³⁻¹⁶⁶ The driving force for copper-free click is the high distortion energy of the cycloalkyne (ΔE_d^{\neq}), which lowers the activation energy (ΔE^{\neq}) required to undergo the cycloaddition by 8.2 kcal/mol.¹⁶⁶ Different cyclooctyne derivatives have different rates of reaction based on substituents that change the polarization of the alkyne bond, thereby changing ΔE^{\neq} of reaction.^{157,167-171}

Spatial and temporal control of copper-free click has been demonstrated by Popik in the use of a cyclopropenone functionalized precursor to dibenzocyclooctyne.¹⁷² Cyclopropenones will readily convert to acetylenes with a single or two-photon excitation.¹⁷³⁻¹⁷⁵ The photodecarbonylation of cyclopropenones to alkynes in solution proceeds quantitatively on the order of picoseconds with high quantum efficiency ($\Phi =$ 0.2-1.0).¹⁷⁶ In masking the reactive alkyne with cyclopropenone, copper free click chemistry can be photo directed (350 nm) to react only where photodecarbonylation forms the reactive cyclooctyne.

Polymer Brush Surface Patterning for Multifunctional Surfaces

Patterned polymer brush interfaces can be fabricated using advanced lithographic techniques in a top-down approach to tailor surface structure and morphology. This has been demonstrated with a variety of advanced techniques such as photolithography, ^{177,178} electron beam lithography^{179,180,181} dip pen lithography,²⁹ nanoshaving,¹⁸² and scratch card lithography.^{183,184} Soft lithographic methods such as microcontact printing, capillary force lithography or nanoimprint lithography have also been used for simplified patterning of polymer brush surfaces. ^{146,185-197}

The formation of multifunctional surfaces allows for the introduction of two or more types of polymer substrates in a well-defined, spatially-oriented area. This can assist in developing specialized structures on surfaces and providing nanoscale and microscale surfaces with chemical specificity. Multicomponent surfaces deal with patterned polymer brush domains that can be formed by two methods: (1) sequential polymerization of patterned surface initiators and/or (2) orthogonal surfaces by postpolymerization modification.

Sequential polymerization involves polymerization, followed by a surface activation step, such as etching of polymer or deposition and activation of a protected initiator species. Several papers have achieved binary functional polymer surfaces by post-polymerization modifications to a polymer brush, followed by a second polymerization to backfill the bare regions.¹⁹⁸⁻²⁰⁰ Sequential polymer brush formation has also been achieved via microcontact printing by the Huck group.²⁰¹ This work went above and beyond the formation of binary functionalized surfaces, to generate up to eight quaternary functionalized surfaces by controlled polymerization (ATRP), passivation of

the initiator end group, printing of a new initiator, and sequential polymerization.²⁰¹ This technique demonstrates high fidelity polymer patterns that are amenable to several types of monomers with different chemical functionality. Patterning polymer brush surfaces can aid in specialized environments for fields such as medicine. Photolithography has been utilized by the Ober group to selectively polymerize polymer domains to template biological growth of rat hippocampal neurons on channels of poly[(2-methyacryloxyloxy)ethyl] trimethylammonium chloride (PMETA) functionalization surrounded by protein-resistant poly(ethylene glycol), which may find utility in the development of neural device coatings.¹⁷⁷

Post-polymerization modification of polymer surfaces allows for site-specific coatings in a one-pot orthogonal functionalization. Specifically, the Patton group used poly(propargyl methacrylate) brushes to functionalize thiol terminated molecules in the radical-mediated thiol-yne click reaction, generated by ultraviolet light (254-470 nm) photopatterning.¹⁷⁸ Gleason and co-workers used capillary force lithography to create nanodomains of amine and alkyne functionalized polymers for the one pot functionalization of NHS esters and azides.¹⁴⁶ As specificity and functionality is demanded of polymeric surfaces on smaller scales, the ability to customize and synthesize tuneable surfaces becomes more and more critical.

Objectives and Outline of this Dissertation

The objectives of this dissertation are as follows: (1) to design a customizable functional polymer brush scaffold for rapid, facile post-polymerization modification, (2) evaluate the functionalization density and efficiency of the functional polymer scaffold, (3) determine the effects of the polymer brush matrix on different post-polymerization modification mechanisms, and (4) pattern multiple chemical functionalities on polymer brush surfaces with high degrees of specificity through top down and bottom up patterning methods. The rest of this dissertation is organized into five chapters.

Chapter 2 describes the synthesis of functional polymer brushes based on the activated ester monomer n-hydroxysuccinimide 4-vinyl benzoate (NHS4VB) by controlled ATRP. Different brush environments are demonstrated in the formation of controlled block copolymers with poly(NHS4VB). Surface coverage of active esters and rate of brush aminolysis with primary amines were determined using aminomethylpyrene and UV-Vis spectroscopy. This chapter was published in *Langmuir*, **2010**, *26*(3) 2136-2143.

Chapter 3 investigates light activation of functionalized polymer brushes for postpolymerization modification. Active ester polymer brushes were functionalized with cyclopropenone-masked dibenzocyclooctynes for the light-activated immobilization of azides using catalyst-free click chemistry. Photodecarbonylation kinetics are rapid and quantitative within the brush layer. Spatially resolved patterned polymer brushes are demonstrated by photopatterning selective areas of the polymer brush and functionalizing with multiple azide containing molecules. This surface immobilization strategy provides a general platform for the generation of multicomponent surfaces with spatially resolved chemical functionality. This chapter was published in *The Journal of the American Chemical Society*, **2010**, *132*, 11024-11026.

Chapter 4 describes the post-polymerization functionalization of poly (NHS4VB) brushes with different alkyl amines containing reactive alkynes that differ in relative rates of activity in azide-alkyne cycloaddition reactions. The pseudo-first order rate constants of "click-type" brush functionalization with azides were determined for both high-energy cyclic alkynes and copper-catalyzed cycloaddition for terminal alkynes. Diffusion limited conditions for polymer brush functionalization were also investigated.

Chapter 5 is devoted to the development of spatially resolved dual functional polymer brush surfaces consisting of poly(NHS4VB) and poly(ethynyl styrene) for the immobilization of amines and azides though aminolysis and copper-catalyzed alkyne/azide cycloaddition. Surface patterning of the brushes is templated from the orthogonal deposition of catechol-functionalized ATRP initiators selectively on titanium oxide in the presence of silicon oxide. Strategies for orthogonal self-assembly of the polymer brushes are discussed and evaluated.

Finally, Chapter 6 summarizes the different projects discussed and the overall impact they will have on future studies.

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CHAPTER 2

HIGH DENSITY SCAFFOLDING OF FUNCTIONAL POLYMER BRUSHES: SURFACE INITIATED ATOM TRANSFER RADICAL POLYMERIZATION OF ACTIVE ESTERS¹

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Abstract

In this article, we describe a method for the polymerization of active esters based on n-hydroxysuccinimide 4-vinyl benzoate (NHS4VB) using surface initiated atom transfer radical polymerization (SI-ATRP). Poly(NHS4VB) brushes have high grafting density, a uniform and smooth morphology, and film thickness increases linearly with reaction time. Block copolymer brushes with 2-hydroxyethyl acrylate, tert-butyl acrylate and styrene were synthesized from surface bound poly(NHS4VB) macroinitiators. The active ester brushes show rapid and quantitative conversion under aminolysis conditions with primary amines, which was studied using grazing incidence attenuated total reflection Fourier transform infrared (GATR-FTIR) and UV-vis spectroscopy. UV-vis was also used to quantify the amount of reactive groups in polymer brush layers of differing thickness. Functionalization of the active ester pendant groups with chromophores containing primary amines showed a linear correlation between the amount of chromophore incorporated into the brush layer and brush thickness. Grafting densities as high as 25.7 nmol/cm² were observed for a 50 nm brush. Block copolymer brushes with buried active ester functional moieties also undergo quantitative conversion with primary amines as confirmed by GATR-FTIR. We discuss the potential of activated ester brushes as universal scaffolds for sensor and microarray surfaces, where the twofold control of functionalizable active ester polymer and block copolymers provides wellordered, tunable microenvironments.

Introduction

Using polymer substrates as the immobilization matrix for affinity biosensors and microarrays has enabled advances in biotechnology, medical diagnostics, drug screening, combinatorial chemistry, and many other areas of interest.¹ Signal amplification techniques, such as fluorescence tagging, can effectively detect very small analyte concentrations, but can interfere with the native microenvironment, conformation of the molecule, and/or binding kinetics.² Specifically, optical biosensors, such as those based on surface plasmon resonance (SPR) have become more commonplace as a real-time, label free technique because no signal amplification of a binding event is necessary.³ This has led to the determination of both thermodynamic and kinetic parameters for many adsorption/hybridization events between biological species.⁴⁻⁶

When designing an immobilization matrix for surface biosensors and microarrays, factors such as the density, activity, orientation and conformation of immobilized probe molecules influence the activity and effectiveness of the binding event.⁷ The matrix must also comply with requirements of minimal non-specific adsorption, and provide stability to environmental changes such as ionic strength, pH, or temperature fluctuations. A degree of specification in the immobilization matrix is necessary for different types of arrays and sensors. A specific example is that surfaces amenable for oligonucleotide arrays are not well suited for use with proteins because of the biophysical and biochemical differences between the two classes of molecules.⁸ Ultimately, techniques that afford coatings with easily tunable parameters provide the most versatility.

Many strategies have been employed to attach polymers to a sensing platform as an immobilization matrix. Among these are the formation of SAMs consisting of low molecular weight polymer chains,9 grafting of polymers with predefined molecular weight to a surface through covalent bonding of a SAM with functional groups on the polymer ("grafting to"),^{10,11} or by synthesizing polymer brushes from the substrate surface ("grafting from") and derivatizing the pendant groups on the polymer.^{12,13} Specifically for SPR substrate chips, carboxymethyl dextrans are often used as a solid support matrix and are commercially available. These carbohydrates are grafted to the surface and derivatized using a sequence of reactions to form activated ester leaving groups along the polymer backbone. These reactive groups facilitate nucleophilic attack with amine terminated moieties and subsequent functionalization of the polymer matrix. Carboxymethyl dextrans and other "grafting to" polymers, used as immobilization matrices, are often hydrophilic to prevent nonspecific adsorption of biological analytes.^{14,15} This hydrophilicity, along with the cross-links that occur between the polymer chains and the surface (and often between the polymer chains themselves), create a hydrogel matrix that is sensitive to pH and ionic strength of its solution environment.¹⁶ This can create an environmental background response that can complicate or even mask binding events that occur at the substrate interface.

N-hydroxysuccinimide (NHS) esters are the most common functional group for biological post-polymerization modification where the conjugation of biomolecules occurs between the amine functional groups on peptides and the activated ester.¹⁷ While NHS esters have a half-life on the order of hours in aqueous systems, their rate of hydrolysis increases in basic environments.¹⁷⁻¹⁹ Hydrolysis and side reactions that can occur in active ester functionalization can be minimized by using a high concentration of the desired amine moiety to be attached or functionalizing in organic solvents in the presence of a proton acceptor.^{17,20}

More recently, the synthesis of polymers containing active ester groups has emerged as a popular strategy for coupling synthetic and biomacromolecules.²¹⁻²⁹ This strategy allows for the straightforward formation of biomolecule/polymer conjugates which can be used to create combinatorial libraries of new biomacromolecules. It has been shown that synthetic polymers coupled to proteins can improve the overall protein stability, solubility, and biocompatibility, and are being utilized in the fields of biotechnology and medicinal research.^{30,31} Controlled polymerizations containing active esters, including the development of block copolymers, have been demonstrated in solution by controlled polymerization techniques such as atom transfer radical polymerization (ATRP),³²⁻³⁴ nitroxide mediated polymerization (NMP),³⁵ ring opening metathesis polymerization (ROMP),³⁶⁻³⁸ and reversible addition-fragmentation chain transfer (RAFT) polymerization.³⁹⁻⁴¹ There are only a few reports of active ester polymers being grafted from solid interfaces, including the formation of active ester polymer brushes using free-radical polymerization of *n*-methacryloyl- β -alanine succinimide ester⁴² and ATRP to polymerize 2-vinyl-4,4-dimethyl azlactone.⁴³

In order to fully develop active ester brushes as a versatile and practical template for sensors and microarrays, the stability of the activated ester brushes and the ability to make controlled block copolymer architectures must be evaluated. By polymerizing activated ester monomers using a controlled polymerization technique, the sensing platform has two facets of control. The NHS ester provides the desired attachment chemistry while the ability to polymerize blocks of different monomers can control the surrounding microenvironment and density of functional groups in the brush. This allows for systematic tuning of properties that are important in array development such as non-specific binding, polymer solubility, and accessibility of the functional moiety to solution analytes. Herein, we report the surface-initiated ATRP of n-hydroxysuccinimide 4-vinyl benzoate (NHS4VB). We demonstrate controlled polymerization through the formation of block copolymers with 2-hydroxyethyl acrylate, tert-butyl acrylate, and styrene in which the NHS block can be either buried or exposed in the brush layer, depending on monomer sequence in the polymerization. To test and quantify the functionalization efficiency, the poly(NHS4VB) brushes are functionalized with primary amine containing chromophores to quantify the amount of activated ester present in the brush architecture and evaluate the conversion efficiency of amide formation along the chain backbone. The NHS active ester polymer brushes have the potential to serve as a universal scaffold for the attachment of both large and small analytes for sensor and microarray surfaces.

Experimental

Materials

All solvents, with the exception of dimethyl sulfoxide (DMSO) and anisole, were distilled from sodium-ketyl (THF) or calcium hydride (toluene and dichloromethane). Anhydrous DMSO and dimethylformamide (DMF) (Drisolv, 99.8% by GC) were purchased from EMD. Anisole was purchased from Alfa Aesar and used as received. Silicon wafers (orientation <100>, native oxide) were purchased from University Wafer. BK7 microscope slides (RI = 1.514) were purchased from VWR. Tert-butyl acrylate, 2-hydroxyethyl acrylate, and styrene were flashed through a basic alumina column to

remove inhibitor. All other chemicals were purchased from Sigma Aldrich and were used as received.

Preparation of Gold Substrates

Microscope slides were cleaned by sonication in isopropanol for 15 minutes each. Slides were rinsed with isopropanol and dried under a stream of argon. Chromium (2 nm) and gold (47 nm) were deposited on the glass slides by thermal evaporation (VE-90, Thermionics Northwest). The slides were then transferred immediately to a plasma vapor deposition chamber (PVD-75, Kurt Lesker), where 3.6 nm of silicon oxide was deposited on top of the gold layer.

Preparation of SI-ATRP Initiator Layers

Silicon wafers, SPR substrates, and glass slides were cut into rectangular pieces (approximately 7 x 20 mm) and sonicated for five minutes each in acetone, ethanol and deionized water (18.2 M Ω). The wafers were dried under a stream of argon, and then subjected to plasma cleaning (Harrick Plasma model PDC-32-G, atmospheric gas, 0.8 mbar. 6.8 Watts) minutes. The initiator. 11-(2-bromo-2for two methyl)propionyloxyundecenyl trichlorosilane, was synthesized following literature procedure.44 The substrates and all dry, degassed reagents were transferred into a nitrogen filled glovebox. One drop of initiator was mixed with 20 mL dry, degassed toluene (approximate concentration 10 mM) and the solution was filtered through a 0.45 µm PTFE filter and poured over the clean silicon wafers in a glass staining jar. After 16 hours, the substrates were removed, rinsed with freshly distilled toluene, and stored in toluene. The self-assembled monolayer was 2.5 nm, measured by ellipsometry. An atomic force microscopy (AFM) topographic image of the monolayer was featureless, with a root mean square (RMS) roughness of 1.2 nm.

Synthesis of NHS4VB

NHS4VB was prepared in a three step procedure from 4-bromobenzaldehyde. Briefly, 4-bromobenzaldehyde was converted to 4-bromostyrene using Wittig chemistry with triphenylphosphine methyl ylide.⁴⁵ 4-Bromostyrene was converted to 4-vinylbenzoic acid through Grignard formation and quenching with CO₂. Finally, coupling of nhydroxysuccinimide with 4-vinylbenzoic acid gave the active ester NHS4VB.^{46,47}

Polymerization of NHS4VB

The initiator substrate and a micro stir bar were placed in a dry, flat bottom Schlenk flask in the glovebox. The NHS4VB monomer (0.662 g, 2.7 mmol) and 0.5 mL DMSO were added to the Schlenk flask. Separately, a stock solution was made that consisted of 0.5 mL DMSO, N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA, 423 μ L, 2.03 mmol), copper (I) bromide (39 mg, 0.27 mmol) and copper(II) chloride (7.26 mg, 0.05 mmol). An aliquot of 93 μ L of the stock solution was added to the Schlenk flask, which was then sealed, brought outside the glovebox and stirred in a 50 °C oil bath for 16 hours. The flask was then opened and exposed to air, and the wafers were rinsed vigorously with DMF and dried under a stream of argon.

Determination of the Polymerization Kinetics for NHS4VB

Six identical polymerizations were assembled as described above, using both silicon wafers and glass slides functionalized with initiator. Polymerization was stopped at different time intervals by removing the flask from the oil bath and opening it to air. The substrates were rinsed thoroughly with DMF and dried under argon. Film thicknesses were characterized for polymerization reactions at 15, 30, 45, 60, 120, and 255 minutes.

Synthesis of Poly(NHS4VB-b-HEA) Brushes

The poly(NHS4VB) wafer and a micro stir bar were placed in a dry, flat bottom Schlenk flask in the glovebox. 2-Hydroxyethyl acrylate (HEA) (0.314 g, 2.7 mmol) and 235 μ L DMSO were added to the Schlenk flask. Separately, a stock solution was made consisting of 0.5 mL DMSO, PMDETA (423 μ L, 2.03 mmol), copper (I) bromide (39 mg, 0.27 mmol) and copper(II) chloride (7.26 mg, 0.05 mmol). 93 μ L of the stock solution was added to the Schlenk flask, which was then sealed, brought outside the glovebox and stirred in a 50 °C oil bath for 16 hours.

Synthesis of Poly(NHS4VB-b-tBA) Brushes

The poly(NHS4VB) wafer and a micro stir bar were placed in a dry, flat bottom Schlenk flask in the glovebox. Tert-butyl acrylate (tBA) (0.346 g, 2.7 mmol) and 265 μ L DMSO were added to the Schlenk flask. Separately, a stock solution was made consisting of 0.5 mL DMSO, PMDETA (423 μ L, 2.03 mmol), copper (I) bromide (39 mg, 0.27 mmol) and copper(II) chloride (7.26 mg, 0.05 mmol). 93 μ L of the stock solution was added to the Schlenk flask, which was then sealed, brought outside the glovebox and stirred in a 50 °C oil bath for 16 hours.

Synthesis of Poly(NHS4VB-b-Styrene) Brushes

The poly(NHS4VB) wafer and a micro stir bar were placed in a dry, flat bottom Schlenk flask in the glovebox. Styrene (0.281 g, 2.7 mmol) and 205 μ L DMSO were added to the Schlenk flask. Separately, a stock solution was made consisting of 0.5 mL DMSO, PMDETA (423 μ L, 2.03 mmol), copper (I) bromide (39 mg, 0.27 mmol) and copper(II) chloride (7.26 mg, 0.05 mmol). 93 μ L of the stock solution was added to the Schlenk flask, which was then sealed, brought outside the glovebox and stirred in a 50 °C oil bath for 16 hours.

Synthesis of Poly(Styrene-b-NHS4VB) Brushes

To an initiator-functionalized silicon wafer in a Schlenk flask, styrene (2.89 g, 27.75 mmol), copper (I) bromide (0.027 g, 0.185 mmol), copper (II) chloride (5 mg, 0.037 mmol), and 2 mL of anisole were added. The flask was bubbled with argon gas one hour to purge the system of oxygen and then PMDETA (291 μ L, 1.40 mmol), which was also purged with argon separately, was added to the reaction. The flask was sealed and put in a 90°C oil bath for 16 hours. The substrate was rinsed thoroughly with anisole, dried under argon, and then polymerized with NHS4VB as described above.

Functionalization of Poly(NHS4VB) Brushes with Primary Amines

Polymer brushes, both the poly(NHS4VB) and relative block copolymer brushes, were converted to functionalized amide derivatives of 4-vinyl benzoic acid using 1aminomethylpyrene (Py-N) and octadecylamine as model compounds (0.12 M in dry DMF) at 40 °C with triethylamine as a proton acceptor.⁴² The glass slides from the polymerization kinetics experiment were functionalized with Py-N and used to quantify the density of functional groups for a given film thickness. Substrates were functionalized with Py-N and removed from the reaction at various times for UV-vis spectroscopy to determine functionalization time for quantitative aminolysis.

Quantification of Active Ester Moiety

The Py-N functionalized glass slides were measured on a UV-vis spectrometer using a slide holder accessory with a sample window area of 19.6 mm². A calibration

curve using Py-N in DMF solution was created with concentrations between 5.0×10^{-6} and 3.3×10^{-5} M. The rate of substitution of Py-N onto the polymer brush was measured by monitoring the appearance of Py-N absorbance with time by UV-vis spectroscopy.

Characterization

Ellipsometry, static contact angle measurements, and surface plasmon resonance were all performed on a Multiskop (Optrel GbR). Null ellipsometry was performed using a HeNe laser at λ = 632.8 nm at 70° and film thicknesses were determined using integrated specific software. At least three spots on each wafer were measured and the thickness was averaged. To obtain thickness values of the samples, a simple box model was employed and a refractive index of n = 1.50 was assumed for all polymer brush layers. SPR measurements were taken in the Kretchmann configuration. AFM images were taken using tapping mode on a Multimode NanoScope IIIa (Digital Instruments/Veeco Metrology) using silicon AFM probes with a 300 kHz resonant frequency and a 40 N/m spring constant. UV-vis spectroscopy was taken on a Varian 50Bio spectrometer. FTIR measurements were taken with a Nicolet Model 6700 with grazing angle attenuated total reflection accessory (GATR) at 256 scans with 4 cm⁻¹ resolution.

Results and Discussion

Synthesis of Active Ester Polymer Brushes

In our initial studies of surface initiated polymerization with active esters, we explored methacrylate-based monomers, such as n-hydroxysuccinimidyl methacrylate (NHSMA), as this monomer has been polymerized by ATRP in solution. When attempting surface initiated polymerization, despite changing reaction temperature and solvent, ratios of Cu(I) and Cu(II), adding sacrificial initiator, and changing monomer concentration, thicknesses greater than 5 nm were not achievable with this monomer. The grazing angle attenuated total reflection spectroscopy (GATR-FTIR) spectrum of poly(NHSMA) brushes showed a significant amount of carboxylic acid present and very little of activated ester. The reason for the reduced film thickness is not completely clear, since the same monomer can be polymerized in a controlled fashion using ATRP in solution, albeit with relatively low molecular weights, less than 50,000 g/mol.⁴⁸ Either partial hydrolysis or catalyst deactivation by complexation with the growing polymer chains are possible reasons for early termination. This has been observed with acrylamides and azlactone polymerizations in solution.^{49,50} For this reason, we turned to the polymerization of 4-vinyl benzoate derivatives, which are known to polymerize with high reaction rates.⁵¹



Figure 2.1. Scheme of SI-ATRP of NHS4VB with two-fold control of polymer architecture. Poly(NHS4VB) can act as a macroinitiator to form block copolymers with a variety of monomers with subsequent derivatization of the NHS containing block.

The reaction scheme for the polymerization of NHS4VB and subsequent functionalization can be seen in Figure 2.1, which depicts a polymer brush matrix with two-fold functionality. First, block copolymers allow for precise spatial placement of functional groups within the brush layer, which can be laterally extended with a block containing different chemical functionality to control the polymer microenvironment. The second dimension of control is the ability to attach new functionality to the polymer brush through conjugation with a primary amine. This allows post-functionalization of the brush surface with a wide variety of molecules that contain functionality incompatible with surface polymerization reactions.

A 16 hour polymerization (50 weight % in DMSO at 50° C) yields films with an average thickness of 50 nm. Without the use of a deactivator (CuCl₂) in the polymerization mixture, the polymer films generated by SI-ATRP had a rougher surface morphology, with an RMS roughness of 2 nm on average for a 1 μ m² area, resulting from

a faster, less controlled rate of polymerization. Early termination is more prevalent due to the larger concentration of propagating radicals. These radicals, in close proximity to each other, can couple or disproportionate prematurely and cease the polymerization. Polymerization with CuCl₂ afforded better control; halogen exchange of the alkyl bromide initiator with CuCl₂ provides for faster initiation relative to the rate of propagation.⁵² The copper (II) also lowers the equilibrium concentration of propagating radicals, which slows propagation and affords better polymer rate control. This resulted in homogeneous film thickness, with deviations of less than 1 nm and an overall uniform, smooth morphology. AFM images of the poly(NHS4VB) brushes prepared with and without Cu(II) are shown in Figure 2.2. In the polymerization with CuCl₂ added to the reaction mixture, the overall brush morphology is featureless with an RMS roughness of 0.5 nm on silicon oxide and 1.5 nm on the gold/silicon oxide surface, on average, for a 1 μm^2 area, which is comparable to the RMS roughness of the substrates themselves.



Figure 2.2. AFM topography images of 20 nm poly(NHS4VB) brushes polymerized with (left) and without CuCl₂ (right).

Chemical functionality of the initiator layer, polymer brush, and derivatized polymer substrate was confirmed by GATR-FTIR. Figure 2.3 shows the stepwise chemical processes required to convert a surface into a reactive ester scaffold. Figure 2.3a verifies the surface attachment of initiator, 11-(2-bromo-2the methyl)propionyloxyundecenyl trichlorosilane, shown by the methyl stretch at 2963 cm⁻¹ and the carbonyl stretch at 1737 cm⁻¹. The spectra of the polymer brush can be seen in Figure 2.3b. The peaks at 1801, 1769, and 1738 cm⁻¹ are carbonyl stretches of the NHS activated ester derivative, while the C-O stretches are visible at 1258 and 1026 cm⁻¹. The peak at 1258 cm⁻¹ is due to the C-N stretch of NHS. Poly(NHS4VB) was then functionalized with aminomethylpyrene (Py-N), indicated by the amide I and II stretches

at 1642 and 1542 cm⁻¹, respectively (Figure 2.3c). A thorough assignment of the vibrations in the initiator and surface bound polymer brushes is highlighted in Table 2.1.



Figure 2.3. GATR-FTIR of the (a) ATRP initiator, (b) poly(NHS4VB) brush, and (c) poly(NHS4VB) brush functionalized with Py-N.

Surface	Frequency (cm ⁻¹)	Assignment ^a	Figure
ATRP initiator	2963	CH ₃ str	2.3a
	2925, 2854	CH ₂ str	
	1737	Assignment " CH_3 str CH_2 str $C=O$ str CH_3 asym str CH defAsym. C-O strSym. C-O strC=O in phase str of imideC=O out of phase str ofC=O str of esterp-substituted aromaticAsym. C-O strC-N str, Amide IIISym. C-O strCH2 strArom C-H strCH2 strAmide Ip-substituted aromAmide IIArom ring strAmide III, CN strNH strArom C-H strCH2 strAmide III, CN strCH2 strAmide III, CN strNH strArom ring strCH3 strCH2 strAmide IIp-substituted aromAmide IIp-substituted aromAmide IIp-substituted aromAmide IIp-substituted aromAmide IIp-substituted aromAmide IICH2 strCH3 sym. DefAmide III, CN strOH strCH2 strC=O out of phase str ofC=O stra such areas fine attr	
	1466	CH ₃ asym str	
	1374	CH def	
	1231	Asym. C-O str	
	1175	Sym. C-O str	
poly(NHS4VB)	1801	C=O in phase str of imide	2.3b
	1769	C=O out of phase str of	
	1738	C=O str of ester	
	1607	p-substituted aromatic	
	1258	Asym. C-O str	
	1205	C-N str, Amide III	
	1026	Sym. C-O str	
poly(NHS4VB)	3300	NH str	2.3c
	3040	Arom C-H str	
functionalized with Py-N	2921, 2853	CH ₂ str	
	1642	Amide I	
	1607	p-substituted arom	
	1532	Amide II	
	1497	Arom ring str	
	1309	Amide III, CN str	
poly(NHS4VB)	3300	NH str	2.8c
functionalized with ODA	3040	Arom C-H str	
	2975	CH ₃ str	
	2921, 2853	CH ₂ str	
	1642	Amide I	
	1607	p-substituted arom	
	1532	Amide II	
	1497	Arom ring str	
	1450	CH ₃ Asym. Str	
	1392, 1367	CH ₃ sym. Def	
	1309	Amide III, CN str	
poly(NHS4VB-b-HEA)	3504, 3372	OH str	2.7a
	2928, 2885	CH ₂ str	
	1772	C=O out of phase str of	
	1731	C=O str	
	1607, 1591	p-subst. arom ring str	

Table 2.1. List of important vibrational modes and mode assignments for the GATR-FTIR spectra of surface attached polymers and block copolymers of poly(NHS4VB).

1450 Sym. str of carboxylate 1396 OH def 1258 Asym. C-0 str 1235, 1175 Sym. C-0 str 1077 Arom CH str 1026 Sym. C-0 str, 1° OH str poly(NHS4VB-b-tBA) 2975 CH ₃ str 2.7b 2925, 2854 CH ₂ str 1727 C=0 str 1633, 1608 p-subst. arom ring str 1551 Asym. Str of carboxylate 1551 Asym. str of carboxylate 1502 Sym. C-0 str 1392, 1367 CH ₃ sym. Def 1225, 149 Sym. C-0 str 1222, 1149 Sym. C-0 str 2924, 2850 CH ₂ str poly(NHS4VB-b-styrene) 3022, 3007 CH st rof arom 2.7c 2924, 2850 CH ₂ str 1737 C=0 out of phase str of 1721 C=0 str 1605 p-subst. arom ring str 1546 Asym. str of carboxylate 1468 Sym. str of carboxylate 1605 p-subst. arom ring str 1546 Asym. str of carboxylate 1605 p-subst. arom ring str 1546		1544	Asym. str of carboxylate	
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1740 C=O str		1740	C=O str	
1605 p-subst. arom ring str		1605	p-subst. arom ring str	
1493, 1452CH in plane bend of arom		1493, 1452	CH in plane bend of arom	
1421, 1373, 1184 Arom C=C ring str		1421, 1373, 1184	Arom C=C ring str	
1262 Asym. C-O str		1262	Asym. C-O str	
1025 Sym. C-O str		1025	Sym. C-O str	

^a Abbreviations: str, stretch; in-plane def, in plane deformation; out plane def, out of plane

deformation; ring def = ring deformation; w= wag; asym, asymmetric; sym, symmetric

In order to utilize the surface bound active ester chains for SPR analysis, the initiator layer must be stable to elevated reaction temperatures, dependent on the conditions for copolymerization and the type of monomer used. This presents a limitation on using a noble metal surface as an SPR platform, due to the thermal lability of the metal-thiol bond used to anchor the initiator layer. Depositing a thin layer of silicon oxide (~4 nm) on top of the metal substrate can circumvent this problem. By utilizing silane chemistry to anchor the initiator species, the SAM is much more tolerant to higher temperatures than the thiol anchor group. We have also found that the gold/silicon oxide substrates are robust for polymerization conditions and do not degrade over time. For example, test substrates of gold/silicon oxide were sonicated for 45 minutes each in dichloromethane, acetone, and water with no degradation or shift in the plasmon resonance angle. For these reasons, poly(NHS4VB) was polymerized on a gold/silicon oxide surface (Figure 2.4), with times and thicknesses identical to polymerization from silicon wafers under the same conditions.



Figure 2.4. Surface plasmon resonance angle scans of the BK7/Cr (1 nm) /Au (47 nm) (green line), ATRP initiator (2.6 nm) on a gold/silicon oxide (3.6 nm) substrate (dashed blue line) and poly(NHS4VB) polymer brush (30 nm) (red line).

The polymer brush is stable to atmospheric conditions over several months, as poly(NHS4VB) substrates, stored in a Petri dish on the bench top, were repeatedly measured using contact angle measurements over a period of 120 days. Figure 2.5 shows the variation in contact angle of the active ester brushes with time. The initial static contact angle of the poly(NHS4VB) was $75^{\circ} \pm 3^{\circ}$ and did not decrease with time, indicating the NHS functionality of the film is preserved and does not hydrolyze when stored in ambient conditions. GATR-FTIR spectra taken after four months of storage in ambient conditions also confirmed the stability, as there was no change in the absorbance
intensity of the characteristic NHS peaks in the spectrum. For comparison, poly(NHS4VB) layers hydrolyzed in aqueous base (50% v/v 0.1 M NaOH and THF) had a contact angle of 34.4° .



Figure 2.5. Time-dependent contact angle measurements of the active ester polymer brushes. The stability test was measured over a period of 120 days.

Kinetics of Polymerization

Figure 2.6 shows the increase in film thickness with time as measured by ellipsometry. The polymer brush thickness increases linearly with time for the first hour in DMSO at 50 °C before plateauing around a thickness of 50 nm. This plateau region, due to early termination reactions, is consistent with other monomers synthesized using SI-ATRP.^{12,53} The concentration of propagating radicals on the tethered chains decreases as the chain ends extend, where the close proximity of radicals cause coupling and disproportionation reactions of the radical chain ends.⁵⁴



Figure 2.6. Kinetic curve for the poly(NHS4VB) brushes. Brush thickness increases linearly with reaction time up to 50 nm. The light grey line is meant to guide the eye.

Block Copolymerizations with Active Ester Polymer Brushes

With the retention of the bromine end group, the poly(NHS4VB) can be chain extended and the controlled nature of SI-ATRP verified. Block copolymers were synthesized using various monomers to explore different backbone functionality and ultimately, tunable microenvironments. Poly(NHS4VB), with a thickness of 25 nm, was used as a macroinitiator for polymerization of 2-hydroxyethylacrylate (HEA). The thickness of the HEA block was 24 nm after 16 hours of polymerization at 50 °C. The GATR-FTIR of the poly(NHS4VB-b-HEA) film is shown in Figure 2.7a. The spectrum shows a combination of vibrations for both NHS4VB and HEA. Specifically, the HEA block is distinguished by the OH stretch and deformation at 3372 and 1396 cm⁻¹, respectively. The ester in the HEA backbone can be identified by the C=O stretch at 1731 cm^{-1} and a C-O stretch at 1175 cm^{-1} . A more thorough peak assignment is shown in Table 2.1.



Figure 2.7. GATR-FTIR spectra of block copolymers grown from poly(NHS4VB): (a) poly(NHS4VB-b-HEA); (b) poly(NHS4VB-b-tBA); (c) poly(NHS4VB-b-styrene); (d) poly(styrene-b-NHS4VB); (e) original poly(NHS4VB) for comparison.

Tertbutyl acrylate (tBA) was also polymerized from a surface bound poly(NHS4VB) brush macroinitiator. In the block copolymer with tBA (Figure 2.7b), the

peaks at 2975, and 1367 cm⁻¹ are consistent with the asymmetric stretch and deformation of the CH₃ in the tert-butyl group of the second block. In addition, the C=O stretch of the ester observed at 1727 cm⁻¹ is due to both the tBA and NHS4VB polymer segments. When a thicker macroinitiator layer was used (poly(NHS4VB) = 50 nm), the thickness of the tBA block was 8.3 nm under the same polymerization conditions. The thinner tBA layer is probably due to the lack of macroinitiator sites because of early termination reactions in the thicker poly(NHS4VB) film.

Block copolymerization with styrene was achieved using a similar 25 nm poly(NHS4VB) macroinitiator. The thickness of the styrene block was 25 nm after 16 hours of polymerization at 90°C. Since both the NHS4VB and second block are styrene based, most of the differences in FTIR spectra between individual polymer and the block copolymer brush (Figure 2.7c) are relative intensity increases of the aromatic stretching bands with respect to the poly(NHS4VB) alone (Figure 2.7e). The aromatic C-H stretch at 3000 cm⁻¹ is much more prevalent in the block copolymer relative to the C-H stretches of the methylene peaks at 2924 cm⁻¹. This is also true for the ring stretch at 1605 cm⁻¹, where the peak not only increases in intensity relative to the alkyl region, but also broadens, due to the slight difference in energy between styrene and the *para*-substituted styrene of the activated ester polymer.

It is interesting to note, when comparing the FTIR spectra of all three block copolymers to the original active ester brush (Figure 2.7e), the intensity of the characteristic active ester bands, especially those between 1738 and 1801 cm⁻¹ are somewhat diminished and two new bands appear around 1550 and 1450 cm⁻¹. These peaks are assigned to the asymmetric and symmetric stretches of a carboxylate group and

denote partial hydrolysis of the active ester during polymerization. Despite exhaustive purification, distillation and removal of water from the starting materials and polymerization glassware, some loss of the NHS ester does occur in block copolymerization, even though the NHS4VB polymer is stable stored in atmospheric conditions. Because of this reason, we investigated the polymerization of NHS4VB from a surface bound styrene macroinitiator. As can be seen from the retention of the C=O stretches at 1802, 1771, and 1740 cm⁻¹ in Figure 2.7d, no degradation of the brush occurs when NHS4VB is polymerized as the second block. These results are somewhat unexpected, since the reaction conditions for the second block are identical to the first, the only difference being the incorporation of a new monomer into the reaction mixture. If conditions were favorable to cause hydrolysis of the ester, then this loss of the NHS ester would be observed for the poly(NHS4VB) brush as well. This, however, does not negate the efficacy of using ATRP for controlled block copolymer synthesis for complex active ester polymer architectures. The poly(NHS4VB-b-styrene) can be regenerated in a solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDC)/NHS, and the FTIR spectrum (Figure 2.8a) shows both the appearance of the active ester peaks, disappearance of COO⁻ stretches at 1550 and 1450 cm⁻¹, and the enhancement of the aromatic ring and C-H stretches. This brush was then functionalized with octadecylamine (ODA) to show that the NHS ester retains its activity towards nucleophilic substitution with primary amines (Figure 2.8b). The complete conversion of NHS ester with ODA, as seen in the formation of the amide I and II stretches at 1542 and 1532 cm⁻¹ and the methyl stretch at 2975 cm⁻¹, indicates that the second block does not prevent adequate diffusion of the analyte into the functional polymer brush.



Figure 2.8. GATR-FTIR spectra of block copolymers of poly(NHS4VB-b-styrene). (a) The EDC/NHS regenerated active ester block copolymer brush of poly(NHS4VB-b-styrene) reacts quantitatively with octadecylamine (b).

Aminolysis of the Active Ester Brushes

Poly(NHS4VB) brushes were reacted with 1-aminomethylpyrene (Py-N) or ODA to quantify both the kinetic and degree of conversion with primary amines. The poly(NHS4VB) polymer brushes, which had an initial contact angle of 75°, were functionalized with Py-N or ODA to give final contact angles of 93.5° and 94.2°, respectively. FTIR spectra taken after functionalization indicate quantitative conversion of the active ester to amide. When comparing the original poly(NHS4VB) films (Figure 2.3b) with a brush functionalized with Py-N (Figure 2.3c), one can see the almost complete disappearance of the imide ring stretches at 1801 and 1769 cm⁻¹ and the ester

carbonyl and carbon/oxygen stretches at 1738, 1258 and 1026 cm⁻¹, while the amide I and amide II bands appear at 1642 and 1542 cm⁻¹. This indicates nearly complete conversion of active ester to amide throughout the 50 nm polymer brush.

Time-dependent UV-vis data was taken to monitor the kinetics of conversion with Py-N. A poly(NHS4VB) brush was subjected to aminolysis conditions (0.12 M Py-N in DMF at 40 °C with triethylamine catalyst) and UV-vis absorbance spectra were recorded at different time intervals. Figure 2.9 plots the absorbance maxima of Py-N (351 nm) with respect to time. The immediate increase in absorbance suggests rapid conversion from the active ester to the amide. The reaction reaches 70% conversion after 6.5 minutes at 40 °C and reaches maximum conversion overnight.



Figure 2.9. Time-dependent functionalization of poly(NHS4VB) with Py-N. The absorbance maxima of Py-N in the brush occurs at 351 nm.

Quantitative Determination of Available Activated Esters

The relationship between thickness and amount of functionalization was also investigated using UV-vis spectroscopy. Further reactions with Py-N were carried out on poly(NHS4VB) brushes grown on glass slides to quantify the number of activated esters in a given area for different brush thicknesses. Separate brush layers of varying thicknesses were subjected to aminolysis with Py-N and the absorbance values measured by UV-vis spectroscopy. It was assumed the Py-N dye reacts with the NHS ester in a 1:1 mole ratio. The extinction coefficient for Py-N was 32,204 cm⁻¹M⁻¹ at 345 nm, measured by a calibration curve in DMF to assure adequate solvation of the Py-N. Using the Beer-Lambert Law, $A = \varepsilon lc$, one can calculate the surface coverage, $d_{surf} = A\varepsilon^{-1}$.⁵⁵ A is absorbance and ε , *l*, and *c* are the extinction coefficient, the thickness of the film, and the concentration of Py-N within the film, respectively. This calculation includes the assumption that the difference in extinction coefficient between Py-N in solution and Py-N in the polymer film is negligible. The linear relationship between polymer brush thickness and NHS concentration is shown in Figure 2.10. The linear fit shown in the plot has a R^2 value of 0.90 with a standard deviation of 3.2 nmol/cm².



Figure 2.10. Surface coverage of Py-N on poly(NHS4VB) with increasing film thickness.

The absence of a plateau indicates that substitution occurs throughout the brush matrix and is independent of film thickness. The correlation illustrates the effective diffusion and substitution of Py-N along the entire polymer brush chain, given the complete linear functionalization for the thicknesses studied.

Upon functionalization with Py-N, the thickness of the polymer brush layer increases due to the increasing molecular mass of the substituent on the attached polymer molecules. The relationship between thickness change and molecular mass in functional polymer brushes has been studied recently by Rühe.⁴² If it is assumed that the grafting density of polymer chains before and after functionalization remains constant, the relationship between film thickness and molar masses can be expressed as:

$$\frac{L_2}{L_1} = \frac{M_2 \rho_1}{M_1 \rho_2}$$
 (Equation 2.1)

where *L* is the polymer brush thickness, *M* and ρ are the molar mass and density of the repeat unit, respectively. The subscripts denote the original (1) and functionalized (2) polymer brush. Since the densities of both polymers are unknown, they can be estimated based on van der Waals radii of molecular fragments as outlined by van Krevelen.⁵⁶ Estimations of polymer density of poly(NHS4VB) and Py-N modified poly(NHS4VB) (Figure 2.11) are shown in Table 2.2. The ratio of film thickness of the functionalized and original brush (L₂/L₁ = 1.70) correlates well with the theoretical value of 1.62, as shown in Table 2.3. Since the experimental ratio is approximately equal to (and slightly greater than) the theoretical value, this further indicates near-quantitative conversion of reactive esters with Py-N.



Figure 2.11. Molecular structure of the repeat unit of poly(NHS4VB) and Py-N functionalized brushes.

Molar Volume Increments ⁵⁶	Poly(NHS4VB)	Poly(NHS4VB)
-CH ₂ -	3 x 16.37	2 x 16.37
-CH- (trivalent)	9.85	9.85
<i>p</i> -substituted phenyl	65.5	65.5
-COO-	23	
-CONH-		21
-N-	6.4	
-CO-	2 x 13.5	
pyrene ⁵⁷		163.6
Total Molar Volume (cm ³ /mol)	180.86	292.69
Molar mass of repeat unit (g/mol)	246.09 (M ₁)	362.75 (M ₂)
Density of repeat unit (g/cm ³)	1.36 (p ₁)	1.24 (p ₂)

Table 2.2. Estimation of polymer density for poly(NHS4VB) and Py-N functionalized brushes.

Table 2.3. Brush thicknesses and thickness ratios of polymers before and after functionalization.

	Poly(NHS4VB)	After Py-N	Ratio
Polymerization time	(L ₁)	(L_2)	L_2/L_1
(min)	Thickness (nm)	Thickness (nm)	
3	10.3	17.0	1.65
7	16.5	24.8	1.50
11.5	26.1	43.8	1.68
20	32.3	61.3	1.90
25	32.5	58.3	1.79
		Average	1.70
		Std. Dev.	0.15

The quantitative functionalization of the brush, along with the linear trend also provides for a tunable concentration of active ester functionality. Using ATRP, polymer thickness (and thus the number of activated esters in the polymer brush) can be tuned with monomer concentration and reaction time. This technique allows for surfaces with a precisely controlled number of active ester sites within a given area. From the UV-vis experiment, a 50 nm poly(NHS4VB) film can be derivatized with 25.7 nmol/cm² of Py-N. This is a nearly three order of magnitude improvement in amount of functional sites compared to SAMs with activated ester end groups.^{58,59} Thus far, the number of reactive sites in these polymer brushes far outnumber analyte binding sites in other macromolecular systems. Xu and coworkers determined that the surface coverage of carboxyl groups in a non-cross-linked carboxymethyl dextran surface was 1.17 nmol/cm², which, assuming quantitative functionalization of the NHS ester, is the number of total functional sites in the polymer matrix.¹⁶ Poly(methacrylic acid) polymer brushes were previously activated and derivatized by the Metters group with NHS and then the amino acid trimer of arginine, glycine and aspartic acid (RGD) with a molecular weight of 382 g/mol. The measurement of RGD bound within the upper 10 nm of the film was determined to be 8.3 nmol/cm^{2.60} Controlled polymerization with NHS4VB not only allows for a greater number of active binding sites, but also controlled polymer architectures for diffusion of solution analytes to interact along the entire length of the polymer brush backbone.

Conclusions

In summary, the surface-initiated atom transfer radical polymerization of a styrenic-based active ester with block copolymerization capability has been demonstrated. Films of poly(NHS4VB) show a linear increase in film thickness with fast reaction times and can be easily re-initiated to form block copolymers with different types of monomers. This versatility leads to different surface architectures, which depend on the co-monomer and functional group coupled to the active ester block. The brushes generated have a uniform, smooth morphology with a high grafting density. Also, post-functionalization of the NHS4VB moiety shows rapid and quantitative conversion, independent of brush thickness, with small molecule amines. The controlled surface-initiated polymerization of active ester polymer brushes and block copolymers will aid in the development of optical sensors and microarrays. With block copolymers, the two-fold control over functionality and polymer microenvironment can template many varieties of macromolecular sensing surfaces that may not be easily polymerized from a functional monomer.

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CHAPTER 3

HIGH DENSITY ORTHOGONAL SURFACE IMMOBILIZATION VIA PHOTOACTIVATED COPPER-FREE CLICK CHEMISTRY¹

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Abstract

Surfaces containing active ester polymer brushes were functionalized with cyclopropenone-masked dibenzocyclooctynes for the light activated immobilization of azides using catalyst-free click chemistry. The photodecarbonylation reaction in the amorphous brush layer is first order for the first 45 seconds with a rate constant of 0.022 s⁻¹. The catalyst-free cycloaddition of surface bound dibenzocyclooctynes proceeds rapidly in the presence of azides under ambient conditions. Photolithography using a shadow-mask was used to demonstrate patterning with multiple azide containing molecules. This surface immobilization strategy provides a general and facile platform for the generation of multicomponent surfaces with spatially resolved chemical functionality.

Introduction

The advancement of engineered particles and surfaces with spatially resolved chemical functionality is of interest to many areas of science and technology including the fabrication of biochips, microfluidic devices, targeted drug delivery, and microelectronic devices. Among several immobilization strategies developed,^{1,2} the alkyne-azide Huisgen 1,3-dipolar cycloaddition is emerging as an ideal coupling approach.²⁻⁴ These "click" reactions are especially appealing for biological attachment due to their quantitative reactivity, small size, and the ability to incorporate azides in biomolecules through native cell machinery.⁵ While conventional copper(I)-catalyzed click chemistry is ideal for many applications,⁶⁻⁸ the cytotoxicity of the Cu catalyst can limit bioorthogonal conjugation. Recently, catalyst-free click reactions have emerged that utilize a reactive cycloalkyne to promote the [3+2] cycloaddition with comparable reaction rates.^{9,10}

Herein, we report the functionalization of activated ester polymer brushes with a cyclopropenone masked dibenzocyclooctyne compound that allows selective immobilization of azido-containing substrates only upon activation with light. A poly(n-hydroxysuccinimide 4-vinyl benzoate) (poly(NHS4VB)) brush coating was chosen as a versatile surface platform because it is densely packed and provides a facile template for post-polymerization functionalization.¹¹ The electrophilic n-hydroxysuccinimide (NHS) ester pendant group allows coupling of a wide variety of functional groups and the controlled nature of surface initiated polymerization allows for homopolymer, copolymer and block copolymer coatings with precise control of functionality and

microenvironment. These surface bound polymer coatings also allow one to decouple sensitive chemistry or multistep monomer synthesis from the polymer brush geometry.

Experimental

Materials

All solvents, with the exception of dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) were used as received. Anhydrous DMSO and DMF (Drisolv, 99.8% by GC) were purchased from EMD. Silicon wafers (orientation <100>, native oxide) were purchased from University Wafer. Quartz microscope slides were purchased from Technical Glass. Oregon Green SE and Rhodamine B were purchased from Invitrogen. All other chemicals were purchased from Sigma Aldrich and were used as received.

Preparation of SI-ATRP Initiator Layers

Silicon wafers and quartz slides were cut into rectangular pieces (approximately 7 x 20 mm) and sonicated for five minutes each in acetone, ethanol and deionized water (18.2 M Ω) water. The wafers were dried under a stream of argon, and then subjected to plasma cleaning (Harrick Plasma model PDC-32-G, atmospheric gas, 0.8 mbar, 6.8 Watts) for two minutes. The initiator, 11-(2-bromo-2-methyl)propionyloxyundecenyl trichlorosilane, was synthesized following literature procedure.¹² The substrates and all dry, degassed reagents were transferred into a nitrogen filled glovebox. One drop of initiator was mixed with 20 mL dry, degassed toluene (approximate concentration 10 mM) and the solution was filtered through a 0.45 μ m PTFE filter and poured over the clean silicon wafers in a glass staining jar. After 16 hours, the substrates were removed, rinsed with freshly distilled toluene, and stored in toluene. The self-assembled

monolayer was 2.5 nm, measured by ellipsometry. An atomic force microscopy (AFM) topographic image of the monolayer was featureless, with an RMS roughness of 1.2 nm.

Synthesis of N-hydroxysuccinimide 4-Vinyl Benzoate (NHS4VB)

NHS4VB was prepared in a three step procedure from 4-bromobenzaldehyde. Briefly, 4-bromobenzaldehyde was converted to 4-bromostyrene using Wittig chemistry with triphenylphosphine methyl ylide.¹³ 4-Bromostyrene was converted to 4-vinylbenzoic acid through Grignard formation and quenching with CO₂. Finally, coupling of nhydroxysuccinimide with 4-vinylbenzoic acid gave the active ester NHS4VB.^{14,15}

Polymerization of NHS4VB

The initiator substrate and a micro stir bar were placed in a dry, flat bottom Schlenk flask in the glovebox. The NHS4VB monomer (0.662 g, 2.7 mmol) and 0.5 mL DMSO were added to the Schlenk flask. Separately, a stock solution was made that consisted of 0.5 mL DMSO, N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA, 423 μ L, 2.03 mmol), copper (I) bromide (39 mg, 0.27 mmol) and copper(II) chloride (7.26 mg, 0.05 mmol). An aliquot of 93 μ L of the stock solution was added to the Schlenk flask, which was then sealed, brought outside the glovebox and stirred in a 50 °C oil bath for 16 hours. The flask was then opened and exposed to air, and the wafers were rinsed vigorously with DMF and dried under a stream of argon.

Synthesis of Cyclopropenone-Amine Conjugate (1)

Diethyl azodicarboxylate (DEAD) (0.275 g, 1.574 mmol) was added to a solution of cyclopropenone $S1^5$ (0.357 g, 1.175 mmol), *t*-butyl 15-hydroxy-4,7,10,13-tetraoxapentadecanoate⁶ (0.491 g, 1.521 mmol), and triphenylphosphine (0.416 g, 1.588 mmol) in tetrahydrofuran (THF) (approximately 15 mL) at room temperature and the

reaction mixture was stirred for 30 min. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (EtOAc : Hex 1:1 to DCM : MeOH 20:1) to provide (0.72 g, 1.18 mmol) of crude ester **S2** (Figure 3.1).

Trifluoroacetic acid (2 mL, 12.98 mmol) was added to a solution of crude ester S2 (0.72 g, 1.18 mmol) in dichloromethane (ca. 5 mL) at room temperature, and the reaction mixture was stirred for approximately 2 hours. The reaction mixture was washed with water, dried over anhydrous MgSO₄, and concentrated. The crude product was dissolved in dichloromethane (3 mL) and NHS (0.18 g, 1.56 mmol) was added, followed by 1ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.298 g, 1.56 mmol) and the reaction mixture was stirred overnight at room temperature and concentrated. The product was purified by chromatography (dichloromethane: MeOH 20:1) to provide 0.49 g (0.737 mmol, 62 % yield over 3 steps) of cyclopropenone-NHS ester S3 as slightly yellow oil (Figure 3.1). ¹H NMR (400 MHz, CDCl₃): 7.90(d, J = 8.8Hz, 2H), 6.86-6.90 (m, 4H), 4.19 (t, 4.4Hz, 2H), 4.02 (t, 6.4Hz, 2H), 3.87 (t, 4.4Hz, 2H), 3.82 (t, J=6.4Hz, 2H), 3.70-3.73 (m, 2H), 3.60-3.68 (m, 10H), 3.31 (d, J=11Hz,2H), 2.87 (t, J=6.4Hz, 2H), 2.81 (s, 4H), 2.60 (d, J=11Hz, 2H), 1.74-1.81 (m, 2H), 1.44-1.53 (m, 2H), 0.97 (t, J= 8Hz Hz, 3H). ¹³C NMR (400 MHz, CDCl₃): 169.13, 166.91, 162.32, 161.85, 153.97, 148.03, 148.01, 142.65, 142.25, 136.02, 135.93, 116.82, 116.59, 116.46, 116.41, 112.61, 112.50, 71.12, 70.94, 70.87, 70.72, 69.73, 68.22, 67.93, 65.94, 37.40, 37.37, 32.38, 32.36, 25.79, 19.41, 14.02. FW calc. C₃₆H₄₄NO₁₁ (M+H⁺): 666.2914, ESI-HRMS: 666.2921.

A solution of cyclopropenone-NHS conjugate **S3** (0.075g, 0.11 mmol) in 3 mL of dichloromethane was added to a solution of 1,8-diamino-3,6-dioxaoctane (0.16 g, 1.1 mmol) in 5 mL of dichloromethane at 0 $^{\circ}$ C. The reaction mixture was warmed to room

temperature and stirred for an hour. The solvent was evaporated and the product was purified using flash chromatography (dichloromethane: MeOH 4:1, + 1% of triethylamine) to yield 49 mg cyclopropenone-amine conjugate **1** as pale yellow oil (62%) (Figure 3.1). ¹H NMR (500 MHz, CDCl₃): 7.92(d, J= 8.5Hz, 2H), 6.87-6.92 (m, 4H), 4.21 (t, J=5Hz, 2H), 4.04 (t, J= 6.5Hz, 2H), 3.88 (t, J=5Hz, 2H), 3.72-3.75 (m, 2H), 3.58-3.69 (m, 16H), 3.55 (t, J=5Hz, 2H), 3.52 (t, J=5Hz, 2H), 3.43-3.47 (m, 2H), 3.30 (d, J=11Hz,2H), 2.87 (t, J=5Hz, 2H), 2.62 (d, J=11Hz, 2H), 2.47 (t, J=6Hz, 2H), 1.77-1.82 (m, 2H),1.48-1.54 (m, 2H), 0.99 (t, J= 7.5 Hz, 3H). ¹³C NMR (500 MHz, CDCl₃): 171.68, 162.36, 161.83, 154.01, 148.06, 148.04, 142.67, 142.20, 136.04, 135.93, 116.85, 116.58, 116.47, 116.39, 112.66, 112.56, 73.34, 71.11, 70.86, 70.85, 70.79, 70.75, 70.60, 70.49, 70.38, 70.09, 69.76, 68.25, 67.92, 67.55, 41.86, 39.39, 37.42, 37.39, 37.20, 31.37, 19.43, 14.04 FW calc. $C_{38}H_{55}N_2O_{10}$ (M+H⁺): 699.3857, ESI-HRMS: 699.3866.



Figure 3.1 Synthetic schemes to prepare cyclopropenone amine (1).

Synthesis of N-(3-azidopropyl)-3',6'-dihydroxy-3-oxo-spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-5-carboxamide (Azido-Fluorescein or Azido-FL)

N,N-diisopropylethylamine (DIEA) (0.073 g, 0.634 mmol) was added to a solution of fluorescein SE (0.1 g, 0.211 mmol) followed by 3-azidopropyl amine (0.025 g, 0.253 mmol) in DMF (2 mL) at room temperature. The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated, and the azido fluorescein was purified by column chromatography (CHCl₃:MeOH:AcOH 100:5:0.5) to provide yellow crystals. The structure of azido-FL is shown in Figure 3.2a.

Synthesis 4-(N-3-azidopropyl)sulfonylamido Lissamine Rhodamine B (Azido-Lissamine Rhodamine B or Azido-RB)

3-Azidopropyl amine (0.049 g, 0.49 mmol) was added to a solution of Lissamine Rhodamine B sulfonyl chloride (0.200 g, 0.347 mmol) in DMF (3 mL) followed by N,N-diisopropylethylamine (0.072 g, 0.628 mmol) and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated and the product was purified by chromatography (CHCl₃:MeOH 15:1) to provide dark red crystals. The structure of azido-RB is shown below (Figure 3.2b).



Figure 3.2. Structures of fluorescent dyes (a) Azido-FL and (b) azido-RB used for polymer brush functionalization.

Functionalization of Poly(NHS4VB) Polymer Brushes with Cyclopropenone Amine

The poly(NHS4VB) was submerged in 1 mL of dry DMF under nitrogen atmosphere. Cyclopropenone amine (30 mg, 0.09 mmol) was added, followed by 10 microliters of triethylamine (0.07 mmol) and allowed to stir in a 40°C oil bath overnight. Substrates were removed, rinsed well with DMF and dried under a stream of nitrogen.

Photoconversion of Cyclopropenone to Dibenzocyclooctyne

The cyclopropenone polymer brushes underwent decarbonylation to form dibenzocyclooctyne using a handheld UV lamp fitted with a Rayonet UV bulb to deliver 350 nm light. The irradiation was carried out at 3.5 mW/cm² for 150 seconds at a distance of 2 cm from the substrate for all thickness, contact angle, and FTIR data. The decarbonylation kinetics was carried out under the same conditions, only irradiating the polymer brush for 1 second intervals from 0 to 150 seconds. For photopatterning, a TEM grid (12 micron pitch) was placed in intimate contact with the polymer brush surface by placing the silicon wafer polymer brush on a slab of poly(dimethylsiloxane) (PDMS). A TEM grid was placed on top of the brush and the whole surface was covered with a

quartz slide. By clamping the quartz slide to the PDMS, no space exists between the grid and the brush surface, giving clean pattern lines. See Figure 3.3 below for a representation of the experimental setup.



Figure 3.3. Schematic of cyclooctyne patterning on the polymer brush by irradiation through a TEM grid.

Copper-Free Click of Dibenzocyclooctyne and Azido-FL/Azido-RB

After irradiation, the brush was submerged in a 5 mg/mL solution of azido-RB at room temperature for 1 hour. The substrate wasys rinsed thoroughly with methanol. The substrate then was immediately irradiated without the TEM mask, again for 150 seconds, and was submerged in a 5 mg/mL solution of azido-FL in methanol for 1 hour. The substrate was removed, rinsed well and dried.

Polymer Brush Characterization

Ellipsometry was performed on a Multiskop (Optrel GbR) instrument. Null ellipsometry was performed using a HeNe laser at $\lambda = 632.8$ nm at 70°, and film thicknesses were determined using integrated specific software. At least three spots on each wafer were measured, and the thickness was averaged. To obtain thickness values of the samples, a simple box model was employed and a refractive index of n = 1.50 was assumed for all polymer brush layers. Static contact angle was measured on a Krüss DSA 100. UV–vis spectroscopy was taken on a Varian 50Bio spectrometer. Fourier transform infrared (FTIR) measurements were taken with a Nicolet model 6700 instrument with a grazing angle attenuated total reflection accessory (GATR) at 256 scans with 4 cm⁻¹ resolution. Fluorescence microscopy pictures were taken using a Zeiss AX10 Observer AX10 inverted microscope with a X-cite Series 120 fluorescent light source and Chroma Technology filters: model 61000 (395, 487 and 555 nm excitation, 450, 517 and 607 nm emission) model 41000 FITC blue filter (480 nm excitation, 535 emission) and model 11000 FITC UV filter (350 nm excitation, > 430 nm emission).

Results and Discussion

Polymer Brush Functionalization with Photoactivated Dibenzocyclooctyne for Catalyst-free Azide Cycloaddition

Figure 3.4 outlines the surface immobilization strategy. First poly(NHS4VB) coatings (125 nm) were prepared using surface-initiated ATRP.¹¹ Cyclopropenone **1** was then immobilized to the brush matrix under aminolysis conditions with quantitative conversion (Figure 3.4a). When irradiated with UV light (350 nm, 3.5 mW/cm²), **1** undergoes rapid decarbonylation to yield the reactive dibenzocyclooctyne **2**. Cyclooctyne

2 can then undergo catalyst-free cycloadditions with azides to yield the triazole-linked conjugate in quantitative yield under ambient conditions (Figure 3.4b). Unexposed cyclopropenone **1** does not react with azides and is thermally stable at 60 $^{\circ}$ C for 12 hours without decomposition.^{16,17} Unreacted **1** can be subsequently decarbonylated by further irradiation with UV light.



Figure 3.4. (a) Attachment of cyclopropenone (1) to poly(NHS4VB) brushes with (b) subsequent photo-activation (2) and functionalization (3) of the polymer brush pendant groups with azide-derived fluorescent dyes azido-FL and azido-RB.

The consecutive functionalization steps of the brush coatings were characterized using ellipsometry, contact angle, and grazing-incidence attenuated total reflectance Fourier

transform infrared spectroscopy (GATR-FTIR). Upon functionalization, polymer brush thickness increases with additional molecular weight of the pendant group and static contact angle measurements confirm anticipated surface wettability changes (Table 3.1).

Pendant Functional Group of Polymer	Brush thickness (nm)	Contact angle (degrees)
N-hydroxysuccinimide	30.29 ± 4	75
cyclopropenone	57.78 ± 4.5	73
dibenzocyclooctyne	56.27 ± 3.9	85
azido-FL	97.0 ± 13.6	79
azido-RB	83.0 ± 5.1	68

 Table 3.1
 Summary of ellipsometric thickness and static contact angle measurements.

Figure 3.5 shows the progression from poly(NHS4VB) brush to the covalent attachment of a fluorescein-azide conjugate (azido-FL) via photo-activated click chemistry. Upon functionalization with **1**, the disappearance of the NHS C=O stretch at 1738, 1769, and 1801 cm⁻¹ is observed along with the appearance of the cyclopropenone C=O stretch at 1846 cm⁻¹ and conjugation of the C=C-C=O at 1608 cm⁻¹ (Figure 3.5a and 3.5b). Upon irradiation, the cyclopropenone C=O stretch disappears yielding cyclooctyne **2** (Figure 3.5c). After the click reaction, the appearance of carboxylic acid stretches at 1757 and 1447 cm⁻¹ (Figure 3.5d) indicate the attachment of the azido-FL (See Table 3.2 for complete peak assignments).



Figure 3.5. Grazing incidence attenuated total reflection Fourier transform infrared spectroscopy (GATR-FTIR) of the original poly(NHS4VB) brush functionalized (a), post-functionalization with cyclopropenone 1 (b), conversion of cyclopropenone to dibenzocyclooctyne 2 (c), and functionalization with azido-FL 3 (d).

Surface	Frequency (cm ⁻¹)	Assignment ^{<i>a</i>}	Figure
Poly(NHS4VB)	1801	C=O in phase str of imide	3.5a
	1769	C=O out of phase str of imide	
	1738	C=O str of ester	
	1607	p-subst. aromatic	
	1258	Asym. C-O str	
	1205	C-N str, Amide III	
	1026	Sym. C-O str	
Cyclopropenone	3017	Aromatic str.	3.5b
functionalized brush	2928, 2858	CH_2 str.	
	1846	C=O str of cyclopropenone	
	1713	C=O str	
	1643	Amide I	
	1608	C=C conjugated to C=O, p	
		subst. aromatic	
	1558	Amide II	
	1340	Amide III, CN str	
	1254	C-O-C str alkyl aryl ether	
	1128, 1096, 1023	C-O-C str	
Dibenzocyclooctyne	3017	Aromatic str.	3.5c
functionalized brush	2928, 2858	CH ₂ str.	
	1713	C=O str.	
	1643	Amide I	
	1608	p subst. aromatic	
	1558	Amide II	
	1254	C-O-C str. alkyl aryl ether	
	1106, 1033	C-O-C str.	
Azido-FL functionalized brush	3017	Aromatic str.	3.5d
	2928, 2858	CH ₂ str.	
	1767, 1710	C=O str	
	1643	Amide I	
	1610	p-subst. arom.	
	1447	OH bend, carboxylic acid	
	1245	C-O-C str, alkyl aryl ether	
	1182	OH str. Ph-OH	
	1039	C-O-C str.	

Table 3.2. List of important vibrational modes and mode assignments for the GATR-FTIR spectra of surface attached polymers of poly(NHS4VB) and derivatives.

^{*a*} Abbreviations: str. = stretch; arom. = aromatic

Photodecarbonylation Kinetics within the Polymer Brush Film

The photodecarbonylation of cyclopropenones to alkynes in solution proceeds quantitatively on the order of picoseconds with high quantum efficiency ($\Phi = 0.2$ -1.0).¹⁷ In the solid crystalline state, it has also been observed that diarylcyclopropenones can undergo decarbonylation with a remarkable quantum efficiency (Φ >1).¹⁸ In order to examine the kinetics of photodecarbonylation in the amorphous brush layers, time dependent UV-vis spectroscopy on quartz substrates was performed. Absorption spectra were recorded at light exposure (350 nm, 3.5 mW/cm²) intervals of 1 second up to 150 seconds and are shown in Figure 3.6. The bands at 335 and 353 nm of cyclopropenone **1** rapidly decrease upon initial exposure to 350 nm light along with the simultaneous emergence of bands at 308 and 326 nm for dibenzocyclooctyne **2**. The spectral observations in the brush layer are consistent with the photodecarbonylation of **1** in solution.¹⁶



Figure 3.6. UV-Vis absorption spectroscopy of the simultaneous decarbonylation of cyclopropenone and formation of cyclooctyne on the polymer brush irradiated with 350 nm UV light. Spectra shown are every 5 seconds until complete disappearance of cyclopropenone after 150 sec.



Figure 3.7. Decay of cyclopropenone absorbance within the polymer brush with time upon UV irradiation (a) and the first order linear plot of the decarbonylation for the first 40 seconds of reaction (b).

The decay of the cyclopropenone absorbance at 353 nm was found to be first order for the first 45 seconds of exposure with a rate constant of 0.022 s⁻¹ (Figure 3.7b). Decarbonylation after 45 seconds deviates from first order behavior even though the photo-conversion is unimolecular. The alteration of decarbonylation kinetics is likely due to non-equivalent sites in the polymer matrix that influence the absorption coefficient and/or quantum yield such as differing states of aggregation or a heterogeneous free volume distribution in the amorphous film.^{19,20} Overall, 95% of the brush decarbonylation is complete within 90 seconds of irradiation with a hand-held UV lamp and quantitative conversion occurs within 150 seconds. No photo-degradation of the polymer substrate or cyclooctyne moieties was observed in the UV-Vis spectra after several minutes of exposure to 350 nm light.

Formation of Spatially-Resolved Functional Polymer Brush Surfaces

further demonstrate the versatility of the photo-click substrates, To cyclopropenone functionalized substrates were irradiated through a shadow-mask to form multicomponent surfaces with spatially resolved chemical functionality. A square patterned transmission electron microscope (TEM) grid (12 micron pitch) was used to mask 1 during irradiation. Substrates were irradiated through the shadow-mask and then immersed in a solution of Lissamine Rhodamine B-azide conjugate (azido-RB). The cycloaddition is complete within 20 minutes and occurred only in the exposed areas, where cyclopropenone groups underwent decarbonylation to generate dibenzocyclooctyne. A subsequent flood irradiation of the substrate liberates the remaining cyclooctyne groups and allows for the functionalization of the protected regions for further click reactions. Azido-FL was then immobilized to generate a multifunctional substrate. Figure 3.8 shows a fluorescence microscopy image of the photopatterned substrates. There is negligible cross-contamination between the two dyes, with excellent segregation between the selectively activated regions.



Figure 3.8. Fluorescence microscope images of a photopatterned surface fabricated by sequential photoactivation of dibenzocyclooctynes: (a) click functionalized azido-RB excited at 550 nm (b) azido-FL excited at 477 nm and (c) both dyes imaged under wide UV excitation (350 nm).

Conclusions

summary, we have demonstrated the surface immobilization of In cyclopropenones undergo photo-induced decarbonylation that to yield dibenzocyclooctynes for catalyst free cycloaddition with azides. The decarbonylation reaction occurs quickly and quantitatively with low power UV irradiation. This photoactivated surface platform allows the creation of multifunctional surfaces with spatially resolved chemical functionality, and can be extended to biological, nanoparticle, and materials science applications.
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CHAPTER 4

INVESTIGATIONS OF POLYMER BRUSH FUNCTIONALIZATION KINETICS: RATE DETERMINATION OF DIFFERENT ALKYNE-AZIDE CYCLOADDITION MECHANISMS¹

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Abstract

chapter, the post-polymerization functionalization of poly(N-In this hydroxysuccinimide 4-vinyl benzoate) brushes with reactive alkynes that differ in relative rates of activity in azide/alkyne cycloaddition reactions is described. The alkyne derived polymer brushes undergo "click"-type cycloadditions with azido containing compounds by two mechanisms: a geometrically energized cyclic octyne (dibenzocyclooctyne amine and aza-dibenzocyclooctyne amine) or a copper catalyzed alkyne/azide coupling reaction (propargyl amine). Using a pseudo-first order limited rate equation, rate constants for dibenzocyclooctyne (DIBO), aza-dibenzocyclooctyne (ADIBO), and propargyl (PPG) polymer brush functionalization with different azides were calculated as $7.7 \times 10^{-4} \text{ s}^{-1}$, 4.4 x 10^{-3} s⁻¹, and 2.0 x 10^{-2} s⁻¹, respectively. Polymer brush click chemistry in these surface bound layers was determined to be slower than the equivalent reactions in solution, while the relative ratio of the reaction rate for DIBO, ADIBO, and PPG functionalization was consistent between solution and the solid state at 1:6.2:28.5. Diffusion of azides into the polymer scaffold did not impact the rate of functionalization as long as the concentration Preliminary studies of dilute solution azide of azide was sufficiently high. functionalization indicate that the diffusion limited regime of brush functionalization impacts a 50 nm polymer brush layer and decreases the pseudo-first order rate by a constant diffusion limited factor of a = 0.233.

Introduction

The incorporation of Sharpless-type click chemistry into post-polymerization modification has recently attracted attention,¹⁻⁶ especially in terms of polymer brush functionalization.^{7,8} Click chemistry has been used previously in the literature to modify both planar and nanostructured interfaces.⁹⁻¹³ The alkyne/azide Huisgen 1,3-dipolar cycloaddition is emerging as an ideal coupling approach, as it is highly selective, tolerant of other functional groups, has straightforward reaction conditions, is compatible with protic, aprotic, and aqueous solvent conditions, and has a rapid rate with high quantitative yields.^{3,14-16} Click chemistry reactions are especially appealing for biological attachment due to their facile incorporation into biomolecules through post-synthetic modification,^{17,18} enzymatic transfer,^{19,20} and azide-modified nutrients for metabolic functionalization.²¹ While conventional copper(I) catalyzed alkyne/azide cycloaddition (CuAAC) is ideal for many applications,²²⁻²⁴ the cytotoxicity of the Cu catalyst can limit bioorthogonal conjugation. Bertozzi and coworkers designed the first alternative to using CuAAC for alkyne/azide cycloaddition by synthesizing a difluoronated cyclooctyne (DIFO) derivative to fluorescently label azide-functionalized glycans in live zebra fish without metal catalysis.²⁵ Diaryl and other substituted high energy cyclic alkynes have also been developed to tailor the reactivity of the copper-free cycloaddition with azides.²⁶⁻²⁹

Recently, catalyst-free click reactions have emerged that utilize a reactive, high energy, "strained" cycloalkyne to promote the [3+2] cycloaddition with comparable reaction rates to copper catalyzed reactions while avoiding toxic metal catalysts.³⁰⁻³⁴ Previous reports have described the catalyst free cycloaddition by the cyclooctyne as

"strain-promoted"; although, a significant amount of energy is not released once ring strain is relieved.³⁵⁻³⁸ Instead, the driving force for copper-free click chemistry is the high distortion energy of the cycloalkyne, which lowers the activation energy required to undergo the cycloaddition by 8.2 kcal/mol.³⁸ Different cyclooctyne derivatives have different rates of reaction based on substituents that change the polarization of the alkyne bond, thereby changing the activation energy.^{28-30,39-41}

Copper-free cycloadditions and CuAAC have been well studied for the functionalization of polymers in solution.¹⁻⁶ The environment of a densely packed polymer brush is much different than a solution, as the surface bound polymer chains are forced into an extended conformation due to excluded volume effects. The extent of polymer brush functionalization for N-hydroxysuccinimide (NHS) esters has been investigated previously.⁴²⁻⁴⁵ In these studies, low molecular weight amines quantitatively bind to NHS esters,^{42,43} but a decrease in penetration depth into the brush is observed for larger, amine-capped polymer analytes in solution.⁴⁴ In order to increase the scope of these alkyne/azide clickable platforms, evaluating the extent of polymer brush Similar to aminolysis, alkyne/azide cycloadditions derivatization is critical.^{44,46-50} proceed quickly with near quantitative yields.^{3,14-16} The different mechanisms of azide/alkyne cycloaddition, however, either by copper catalyzed reaction or use of a high energy cyclic alkyne, require individual evaluation in a confined polymer brush environment. In order to incorporate CuAAC and catalyst-free cycloadditions as methods to develop functional polymer brush platforms, the extent of functionalization and rate of post-polymerization modification must be well understood in a surface confined environment.

Previously, it has been observed that poly(N-hydroxysuccinimide 4-vinyl benzoate) (poly(NHS4VB)) polymer brush scaffolds can easily incorporate alkyne functionality into a densely packed, three-dimensional macromonolayer through aminolysis with alkyne functionalized amines.⁴⁵ This derivatization generates alkyne groups along the backbone of the polymer brush, which can be used to couple azides to the surface with high density. In the current study, the relative rates of click reactions on polymer brush scaffolds that contain different functional alkyne pendant groups, dibenzocyclooctyne (DIBO), aza-dibenzocyclooctyne (ADIBO), and a terminal alkyne group (propargyl, PPG) are investigated. DIBO and ADIBO undergo cycloaddition without a metal catalyst while the alkyne pendant group requires copper(I) catalyst. The rates of polymer brush functionalization are compared to the reaction in solution, as well as the relative rates of azide coupling on the brush scaffold between three different These investigations will establish the extent of polymer brush alkynes. functionalization, determine the rate of azide cycloaddition reactions, and elucidate the influence of the polymer brush environment for azide post-polymerization modification.

Experimental

Materials

All solvents, with the exception of dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), chloroform (CHCl₃), and methanol (MeOH) were distilled from sodium-ketyl (THF) or calcium hydride (toluene and dichloromethane (DCM)). Anhydrous DMSO and DMF (Drisolv, 99.8% by GC) were purchased from EMD. Methanol and chloroform were purchased from VWR (ACS grade) and used as received. Silicon wafers (orientation <100>, native oxide) were purchased from University Wafer.

Quartz microscope slides were purchased from Technical Glass Products. N,N',N',N'',N'' pentamethyldiethylenetriamine (PMDETA) was purchased from Alfa Aesar and distilled prior to use. All other chemicals were purchased from Sigma Aldrich and used as received.

Preparation of SI-ATRP Initiator Layers

Silicon wafers and quartz slides were cut into square pieces (approximately 1 cm^2) and sonicated for five minutes each in acetone, ethanol, and deionized water (18.2) M Ω). The wafers were dried under a stream of nitrogen, and then subjected to argon plasma cleaning (Harrick Plasma model PDC-32-G, 0.8 mbar, 18 Watts) for two minutes. The initiator. 11-(2-bromo-2-methyl)propionyloxyundecenyl trichlorosilane, was synthesized following literature procedures.⁵¹ The substrates and all dry, degassed reagents were transferred into a nitrogen filled glovebox. One drop of initiator was mixed with 20 mL dry, degassed toluene (approximate concentration 10 mM), and the solution was filtered through a 0.45 µm poly(tetrafluoroethylene) (PTFE) filter and poured over the clean substrates in a glass slide holder. After 16 hours, the substrates were removed, rinsed with freshly distilled toluene, and stored in toluene. The selfassembled monolayer was 2.5 nm, measured by ellipsometry. An atomic force microscopy (AFM) topographic image of the monolayer was featureless, with a root mean square (RMS) roughness of 1.2 nm.

Synthesis of N-hydroxysuccinimide 4-Vinyl Benzoate (NHS4VB)

NHS4VB was prepared in a three step procedure from 4-bromobenzaldehyde. Briefly, 4-bromobenzaldehyde was converted to 4-bromostyrene using Wittig chemistry with triphenylphosphine methyl ylide.⁵² 4-Bromostyrene was converted to 4-vinylbenzoic acid through Grignard formation and quenching with CO₂. Finally, coupling of N-hydroxysuccinimide with 4-vinylbenzoic acid gave the active ester NHS4VB.^{53,54}

Polymerization of NHS4VB

The initiator substrates, one quartz and one silicon wafer, were placed in a dry, flat bottom Schlenk flask in a glovebox. The NHS4VB monomer (1.65 g, 6.75 mmol) and 1.2 mL of DMSO were added to the Schlenk flask. Separately, a stock solution was made that consisted of 0.5 mL of DMSO, PMDETA (423 μ L, 2.03 mmol), copper(I) bromide (39 mg, 0.27 mmol) and copper(II) chloride (7.26 mg, 0.05 mmol). An aliquot of 230 μ L of the stock solution was added to the Schlenk flask, which was then sealed, brought outside the glovebox and stirred in a 50 °C oil bath for 1 hour to yield a polymer brush of approximately 50 nm thickness. The flask was then opened to air, and the wafers were rinsed thoroughly with DMF and dried under a stream of nitrogen.

Synthesis of DIBO Amine

The cyclopropenone amine precursor was prepared following procedures described in the literature.²⁶ A solution of cyclopropenone (0.1 g, 0.298 mmol) in methanol (10 mL, 2.98×10^{-2} M) was irradiated (15 bulbs, 8 W each, 350 nm) for 5 min at room temperature. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (MeOH:DCM 1:20) to provide dibenzocyclooctyne amine (0.076 g, 83%) as a slightly yellow oil. ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.24 – 7.17 (dd, *J* = 8.3, 5.2 Hz, 2H), 6.90 – 6.86 (m, 2H), 6.79 – 6.72 (dt, *J* = 8.3, 2.3 Hz, 2H), 4.09 – 3.99 (t, *J* = 6.1 Hz, 2H), 3.83 (s, 3H), 2.95 – 2.87 (t, *J* = 6.8 Hz, 2H), 2.51 – 2.35 (m, 2H), 1.98 – 1.86 (p, *J* = 6.5 Hz, 2H). ¹³C NMR (101 MHz, CDCl₃) δ (ppm) 159.26, 158.67, 155.09, 126.90, 116.89, 116.38, 112.02, 111.49, 110.62,

66.15, 55.54, 36.43, 36.86, 33.17. FW calc. ($[C_{20}H_{21}NO_2]H^+$): 308.1645, ESI-HRMS: 308.1644.

Synthesis of ADIBO Amine

The ADIBO amine was prepared according to literature procedure.²⁹

Functionalization of Poly(NHS4VB) Brushes with Reactive Alkyne Primary Amines

Poly(NHS4VB) brushes were converted to functionalized amide derivatives of 4vinyl benzoic acid using DIBO amine, ADIBO amine, and propargyl (PPG) amine (36.2 mM in dry DMF) at 40 $^{\circ}$ C for two hours with triethylamine as a proton acceptor.⁴³

Synthesis 4-(N-3-Azidopropyl)sulfonylamido Lissamine Rhodamine B (Azido-RB)

3-Azidopropyl amine (0.049 g, 0.49 mmol) was added to a solution of Lissamine rhodamine B sulfonyl chloride (0.200 g, 0.347 mmol) in DMF (3 mL) followed by the addition of N,N-diisopropylethylamine (0.072 g, 0.628 mmol), and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated and the product was purified by chromatography (CHCl₃:MeOH 15:1) to provide dark red crystals.

Determination of Polymer Brush Functionalization Kinetics of Copper-Free Click Alkynes: DIBO and ADIBO by UV-vis Spectroscopy

A quartz slide with a cyclooctyne-functionalized polymer brush, either DIBO or ADIBO, was submerged in a 40 mM solution of azido-RB in methanol:DMF (approximately 10:1, v/v) for several seconds, removed, immediately rinsed thoroughly with methanol, and dried under a stream of nitrogen. A UV-vis spectrum was then recorded between 190 and 700 nm. This cycle was repeated several times until the absorbance of azido-RB attached to the brush no longer increased. An identical polymer

brush on a silicon wafer was placed in the azido-RB solution for an equivalent amount of time as the quartz substrate to obtain an accurate measure of film thickness change, contact angle change, and to confirm chemical functionality by grazing angle attenuated total reflection Fourier transform infrared spectroscopy (GATR-FTIR). The kinetics experiment was repeated with a dilute solution of azido-RB in MeOH:DMF (500 μ M) using an identical ADIBO-functionalized brush as directed above in order to determine the diffusion limited rate of brush functionalization.

Determination of Polymer Brush Functionalization Kinetics of Alkyne Polymer Brushes via Copper Alkyne/Azide Cycloaddition (CuAAC) by UV-vis Spectroscopy

A flat bottom Schlenk flask was placed under an argon blanket, and a 1 mL solution of 40 mM azido-RB (MeOH:DMF, 10:1) was added and allowed to equilibrate. Separately, a stock solution of copper(I) bromide (28.7 mg, 0.2 mmol), PMDETA (41.8 μ L, 0.2 mmol), and sodium ascorbate (79.2 g, 0.4 mmol) was prepared in a 10 mL DMF:water solution (9:1, v/v) and degassed with argon for 1 hour. A 100 μ L aliquot of the stock solution was added to the azido-RB solution and stirred to equilibrate the solution. An alkyne-functionalized polymer brush on quartz was immersed in the solution under a blanket of argon gas for 10 seconds. The quartz slide was removed, thoroughly rinsed with methanol, and dried under nitrogen gas. A UV-vis spectrum was taken from 190 to 700 nm to observe the increase in absorbance of the covalently–bound azide dye. This was repeated several times until absorbance measurements no longer changed after additional azido-RB deposition cycles. An identical polymer brush on a silicon wafer was placed in the azido-RB solution for the total amount of time the quartz substrate was in the solution to determine an accurate measure of film thickness change,

contact angle change, and to confirm functional groups on the polymer brush surface using GATR-FTIR.

Determination of Kinetic Rate of Corresponding DIBO and ADIBO in Solution

A 1:1 molar ratio of cyclooctyne and benzyl azide were combined in a quartz cell in a UV-vis spectrometer fixed at 315 nm for ADIBO and 326 nm for DIBO. Kinetics were measured using the disappearance of the cyclooctyne absorption with time and fitted using software (Origin 8.0) to calculate the pseudo-first order and second order rate constants.

Characterization

Spectroscopic ellipsometry was performed on a J. A. Woollam M-2000V spectroscopic ellipsometer with a white light source at 65°, 70°, and 75° angles of incidence to the silicon wafer normal. Delta (Δ) and psi (Ψ) were measured as a function of wavelength between 400 and 1000 nm. Polymer film thicknesses were determined using a three layer model (Si, SiO₂, and polymer), where the polymer's refractive index was modeled using the Cauchy equation (Equation 4.1),

$$n = a + \frac{b}{\lambda^2}$$
 (Equation 4.1)

where n is the refractive index, a and b are fitted parameters, and λ is the wavelength of light. The software allowed for simultaneous modeling of film thickness and refractive index for the polymer. Fitted parameters a and b were computed for each film with an average value for all samples being 1.47 and 0.009, respectively. Thickness measurements for all azido-RB functionalized brushes were also measured by profilometry (Veeco Dektak 150) because the rhodamine B absorbs within the wavelength range, distorting the models fit. Static contact angle measurements were

taken on a Krüss DSA 100 using a 1 μ L drop. UV-vis spectroscopy was taken on a Varian 50 Bio spectrometer. FTIR measurements were taken with a Nicolet Model 6700 with a grazing angle attenuated total reflection accessory (GATR) at 128 scans with 4 cm⁻¹ resolution. UV-vis kinetic measurements were analyzed at λ_{max} of azido-RB dye (570 nm) using linear regression software in Matlab (MathWorks) for pseudo-first order limited kinetics.

Results and Discussion

Aminolysis with of Poly(NHS4VB) Brushes with Reactive Alkynes

Our previous studies demonstrated that the controlled polymerization of NHS4VB by atom transfer radical polymerization (ATRP) affords a linear increase in brush thickness with time for the first hour of polymerization and has an overall homogenous morphology with a RMS surface roughness of less than 2 nm.⁴² Activated ester polymer brushes undergo quantitative functionalization with small molecules, such as primary amines, within an hour of reaction time.^{42,43} In this study, poly(NHS4VB) brushes were functionalized with primary amines that contained either a dibenzocyclooctyne with high distortion energy used for copper-free click chemistry, or a terminal alkyne for conventional copper alkyne/azide cycloaddition (CuAAC).

Poly(NHS4VB) brushes were functionalized with DIBO, ADIBO, and PPG amines. A depiction of DIBO, ADIBO, and PPG functionalized brushes can be seen in Figures 4.1a and 4.1b. An increase in brush thickness of approximately 60% was observed for the cyclooctynes (71 nm to 110 nm for DIBO, 56.8 nm to 89.8 nm for ADIBO). For the low molecular weight propargyl group, the brush thickness decreased by 24%, where film thickness went from 50 nm to 38.3 nm due to the smaller molecular

weight and molar volume of PPG relative to the NHS ester. A summary of brush thicknesses and contact angles for all functionalized films can be found in Table 4.1. The DIBO and propargyl brushes are more hydrophobic upon functionalization, increasing static contact angle measurements by 10 degrees. The ADIBO brushes, however, had the same contact angle as the original poly(NHS4VB) brush. The nitrogen atom in the cyclooctyne ring of ADIBO increases the hydrophilicity of ADIBO⁵⁵ and accounts for the lower contact angle change relative to the DIBO functionalized brushes.



Figure 4.1. (a) Depiction of poly(NHS4VB) functionalized with alkyne-containing amines: DIBO (1), ADIBO (2), and PPG (3). (b) Alkyne/Azide cycloaddition on the polymer brush backbone using energetically-promoted copper-free (4 and 5) cycloaddition and CuAAC (6) to form the triazole.

Sample	Brush thickness (nm)	Contact angle (degrees)
Poly(NHS4VB)	71.0	65
DIBO-functionalized	110.0	79
Azido-RB functionalized	175.9*	78
Poly(NHS4VB)	56.8	65
ADIBO-functionalized	89.8	64
Azido-RB functionalized	199.3*	74
Poly(NHS4VB)	50.0	65
PPG-functionalized	38.3	72
Azido-RB functionalized	96.3*	62

Table 4.1: Summary of thicknesses and contact angles for all polymer brushes on silicon

 wafers. *Brush thicknesses were measured using surface profilometry.

Characterization of Alkyne Functionalized Brushes Before and After Reaction with Azido-RB

The DIBO, ADIBO, and PPG-containing polymer brushes were functionalized with azido-rhodamine B conjugate (azido-RB, Figure 4.1b) and monitored by UV-vis spectroscopy until the reaction was complete. The contact angles for azido-RB clicked brushes are shown in Table 4.1. The static contact angle for DIBO brushes functionalized with azido-RB decreases slightly from 79° to 78°. The contact angle for ADIBO increases from 64° to 74° upon functionalization with azido-RB. For the PPG brushes, the contact angle decreases from 72° to 62° upon functionalization. While the exact cause of the difference in contact angle of the resulting films is not known, we speculate that the overall contribution of the zwitterionic Lissamine-rhodamine B to the

surface energy of the film is greater with the lower molecular weight PPG than with the DIBO or ADIBO substituents, as the measured contact angle is similar to the contact angle of rhodamine B monolayers, at 69° .⁵⁶

The large increase in film thicknesses of the brushes observed after functionalization is due to the increase in the molar mass of the substituent upon azido-RB conjugation. The RB-functionalized brush thicknesses were measured by spectroscopic ellipsometry but were difficult to model due to the strong absorbance of the azido-RB dye within the wavelength range used. Therefore, brush thicknesses for azido-RB functionalized brushes in Table 4.1 were confirmed using surface profilometry. The relationship between thickness change and molecular weight in covalently bound polymer brush systems has been studied by Rühe^{43,44} and can be applied to the poly(NHS4VB) brushes in this study.^{42,57} If it is assumed that the grafting density of polymer chains before and after functionalization remains constant, the relationship between film thicknesses and molar masses can be expressed as

$$\frac{L_2}{L_1} = \frac{M_2 \rho_1}{M_1 \rho_2}$$
 (Equation 4.2)

where *L* is the polymer brush thickness, and *M* and ρ are the molar mass and bulk density of the repeat unit, respectively. The subscripts denote the original (1) and functionalized (2) polymer brushes. While the bulk densities of both alkyne-functionalized and azido-RB functionalized polymers are unknown, their values can be estimated based on van der Waals radii of molecular fragments as outlined by van Krevelen.⁵⁸ Unfortunately, the molar volume increments referenced in the literature do not account for the geometric configurations of the strained dibenzocyclooctynes DIBO and ADIBO, and yield an overestimation of molar volume. The molecular weights of DIBO and ADIBO amine are relatively small (307 g/mol and 276.2 g/mol), and conjugated systems of similar molecular weight (for example, 1-aminomethylpyrene with a molar mass of 267.8 g/mol) undergo quantitative conversion with active esters throughout the entire polymer backbone of the tethered polymer chains.^{42,43} With this low molecular weight pendant group and spectroscopic evidence (complete disappearance of the C=O stretch of NHS side chain using GATR-FTIR in Figures 4.2-4.4), quantitative conversion of the active NHS ester to both DIBO and ADIBO occurs. The density and volume of DIBO and ADIBO functionalized brushes, therefore, can be more accurately approximated using the measured thickness ratio L_2/L_1 for the cyclooctyne (L₂) functionalization of poly(NHS4VB) (L₁). Using these values, the thickness changes upon azido-RB functionalization can be estimated and used to calculate the degree of conversion for the copper-free click reaction from the measured values. A summary of molecular weights, calculated molar volumes, and densities for azido-RB functionalized substrates can be found in Table 4.2. The theoretical and experimental thickness ratios for azido-RB functionalized brushes can be found in Table 4.3. The percent functionalization of the brushes with the azide dye varies between 60% and 86% among the three different alkyne Unlike with the activated esters and primary amines, quantitative systems. functionalization of the brushes with the RB dye by thickness changes is not observed.

Table 4.2.	Molar	volume	and	density	values	for	DIBO,	ADIBO,	and	alkyne	brushes
before and a	fter azio	do-RB fi	ıncti	onalizati	ion.						

Molar Volume Increments ^a	Total Molar Volume	Molar Mass of	Density of Repeat
(cm ³ /mol)	(mol/cm ³)	Repeat Unit, M (g/mol)	Unit, ρ (g/cm ³)
Poly(NHS4VB)	180.86	246.09	1.36
Poly(NHS4VB) post DIBO	280.29 ^b	451.31	1.61
Poly(NHS4VB) post ADIBO	286.08 ^b	406.29	1.42
Poly(NHS4VB) post PPG	140.59	185.13	1.317
DIBO brushes post RB azide	727.60 ^c	1057.41	1.453
ADIBO brushes post azido-RB	733.39°	1012.39	1.38
Alkyne brushes post azido-RB	596.40 ^c	791.23	1.327

^a Molar volumes calculated by van Krevelen, D. W., *Properties of Polymers: Their Estimation and Correlation with Chemical Structure*. 2nd ed.; Elsevier Scientific: New York, 1976, Unless otherwise noted. ^b calculated from thickness ratios between cyclooctyne and poly(NHS4VB). ^C calculated by van Krevelen and using a molar volume of Rhodamine B = 372.4 mol/cm³.

	L_2/L_1 measured	L_2/L_1 calculated	Percent sites functionalized
DIBO	1.60	2.59	61%
ADIBO	2.22	2.56	86%
PPG	2.51	4.24	60%

Table 4.3. Thickness ratios for both experimental and theoretical changes of DIBO, ADIBO, and PPG brushes before (L_1) and after (L_2) azide functionalization.

Chemical functionality of the active ester polymer brush, derivatization with DIBO amine, and attachment of azido-RB to the polymer brush were confirmed by GATR-FTIR. Figure 4.2 shows the FTIR spectra characterizing the chemical processes required to convert an (a) active ester surface into an (c) azido functionalized scaffold by (b) DIBO functionalization. The spectra of the polymer brush functionalized with DIBO can be seen in Figure 4.2b. The peaks at 1801, 1769, and 1738 cm⁻¹ are assigned to the carbonyl stretches of the NHS activated ester, while the C-O stretches from DIBO are visible at 1233 and 1035 cm⁻¹. The peak at 2258 cm⁻¹ is due to the C=C stretch of the cyclooctyne. Functionalization of the DIBO brush was indicated by the triazole absorbances at 1538 and 1262 cm⁻¹, representing the C=N and N=N stretches, respectively (Figure 4.2c). The sulfonate on rhodamine B is also visible at 1179 cm⁻¹. A thorough assignment of the vibrations in the original and functionalized polymer brushes are highlighted in Table 4.4.



Figure 4.2. GATR-FTIR spectra of (a) poly(NHS4VB), (b) DIBO amine functionalized brushes, and (c) azido-RB functionalized brushes. DIBO and azido-RB brush spectra are shown at 5 times the absorbance intensity.

Surface	Frequency (cm ⁻¹)	Assignment ^a	Figure
poly(NHS4VB)	1801	C=O in phase str of imide	4.2a, 4.3a, 4.4a
	1769	C=O out of phase str of imide	
	1738	C=O str of ester	
	1607	p-substituted aromatic	
	1258	Asym. C-O str	
	1205	C-N str, Amide III	
	1026	Sym. C-O str	
poly(NHS4VB)	3555	NH str	4.2b
	3010	Arom C-H str	
functionalized with	2932, 2825	CH ₂ str	
DIBO amine	2258	C≡C str	
	1660	Amide I	
	1613	p-substituted arom	
	1563	Amide II	
	1489, 1432	Arom ring str	
	1264	Amide III, CN str	
	1233,1035	C-O str	
DIBO functionalized	3566	NH str	4.2c
1 1 1	3067	Arom C-H str	
polymer brushes	2960, 2867	CH ₃ str	
coupled with azido-	2926, 2859	CH ₂ str	
	2254	Residual C≡C str	
RB	1633	Amide I	
	1596	C=N str of triazole	
	1554	Amide II	
	1262	N=N str of triazole	
	1179	S=O str	
poly(NHS4VB)	3544	NH str	4.3b
f	3011	Arom C-H str	
functionalized with	2931, 2879	CH ₂ str	
ADIBO amine	2243	C≡C str	
	1654	Amide I & Arom-NH-C=O str	
	1607	p-substituted arom	
	1538	Amide II	
	1500, 1444	Arom ring str	
	1289	Amide III, CN str	
ADIBO	3541	NH str	4.3c
functionalized	3010	Arom C-H str	
runcuonanzed	2974, 2826	CH ₂ str	
	2248	Residual $C \equiv C$ str	

Table 4.4. List of important vibrational modes and mode assignments for the GATR-FTIR spectra of surface attached poly(NHS4VB) and reactive derivatives.

polymer brushes	1643	Arom-NH-C=O str	
	1588	C=N str of triazole	
coupled with azido-	1550	Amide II	
RB	1241	N=N str of triazole	
	1178	S=O str	
Poly (NHS4VB)	3555	NH str	4.4b
	3280	C≡CH str	
functionalized with	2960, 2891	CH ₂ str	
propargyl amine	2216	C≡C str	
FF 87	1640	Amide I	
	1607	p-substituted arom	
	1535	Amide II	
	1491	Arom ring str	
	1309	Amide III, CN str	
Alkyne	3567	NH str	4.4c
с: 1: 1	3068	Arom C-H str	
functionalized	2955, 2879	CH ₃ str	
brushes coupled	2927, 2826	CH ₂ str	
Ţ	2257	Residual C≡C str	
with azido-RB	1650	Amide I	
	1594	C=N str of triazole	
	1534	Amide II	
	1258	N=N str of triazole	
	1176	S=O str	

^{*a*} Abbreviations: str, stretch; arom, aromatic

Figure 4.3 shows the functionalization of the (a) active ester brushes with (b) ADIBO amine, followed by functionalization with (c) azido-RB. Prominent absorbances for ADIBO are the amide in the cyclooctyne ring at 1643 cm⁻¹ and the C=C stretch of the cyclooctyne at 2243 cm⁻¹. Azido-RB functionalization is again observed by the triazole absorbances at 1538 and 1241 cm⁻¹ and the sulfonate at 1179 cm⁻¹.



Figure 4.3. GATR-FTIR of (a) poly(NHS4VB) brushes functionalized with (b) ADIBO amine then (c) azido-RB. ADIBO is shown at 3 times the absorbance intensity.

Propargyl (PPG) functionalization and derivatization is shown in Figure 4.4. A terminal alkyne stretch is visible at 3280 cm⁻¹, as well as amide I, II, and III bands at 1640, 1535, and 1309 cm⁻¹ (Figure 4.4b). Functionalization with azido-RB was confirmed by loss of the terminal alkyne band and the presence of additional absorbances at 1594 and 1258 cm⁻¹ assigned to the triazole and the absorbances at 1176 cm⁻¹ for the sulfonate group (Figure 4.4c).



Figure 4.4. GATR-FTIR spectra of (a) poly(NHS4VB), (b) active ester brush functionalized with PPG amine, and (c) subsequent derivatization with azido-RB. PPG and azido-RB brush spectra are shown at 5 times the absorbance intensity.

Time-Dependent Azido-RB Functionalization of Polymer Brushes Using Both Copper-Free and CuAAC Click Chemistries

Polymer brush functionalization with azido-RB was monitored by UV-vis spectroscopy, in which several spectra were taken after submerging the alkyne polymer brushes in 40 mM azido-RB for various time intervals. This method allows for the monitoring of the absorbance of the dye at $\lambda_{max} \approx 570$ nm, which indicates the alkyne/azide cycloaddition onto the brush. The UV-vis absorbance spectra of the functionalization of DIBO brushes can be seen in Figure 4.5. The DIBO absorbance decreases with time at 306 nm and 325 nm. Triazole formation, which is indicated by the immobilization of RB dye into the brush layers, is indirectly observed by the increasing

absorbance with time at 575 nm. The spectra indicate that brush functionalization is complete after 60 minutes of reaction time, where absorbances of DIBO and RB are unchanging.



Figure 4.5. UV-vis absorbance spectra of DIBO brush functionalization with time. Functionalization is complete after 60 minutes of reaction.

Copper-free cycloaddition of azido-RB and the highly energized cyclooctyne were also recorded with ADIBO functionalized polymer brushes, which can be seen in Figure 4.6. Absorbances of ADIBO at 297 nm and 316 nm decrease with time and shift to a shorter wavelength at 295 nm and 314 nm, respectively. Due to this shift in absorbance, it is difficult to observe the change in absorbance at the λ_{max} at a fixed

wavelength. For consistency, the increase in RB dye absorbance was used for rate constant calculations for ADIBO, DIBO, and PPG. The absorbance of azido-RB on the functionalized polymer brush was observed at 575 nm. The absorbances of ADIBO and RB remain constant after 15 minutes, indicating that the reaction is complete.



Figure 4.6. UV-vis spectra of ADIBO polymer brushes undergoing catalyst free cycloaddition with azido-RB dye. Functionalization is complete after 15 minutes of reaction.

Time dependent functionalization of PPG derivatized polymer brushes with azido-RB using conventional copper catalyzed alkyne/azide cycloaddition was monitored by UV-vis spectroscopy (Figure 4.7). The PPG functionalized polymer brush shows two absorbance bands at 204 nm and 244 nm, which encompass both the absorbance of the alkyne and the styrenic polymer backbone. A measurable change in these absorbances is not observed. There is a broad, weak band at 330 nm which can be attributed to triazole formation. Absorbance of the RB attached to the polymer brush can is observed at 577 nm and increases with reaction time for two minutes, and then remains constant.



Figure 4.7. UV-vis absorption spectra of PPG derivatized polymer brushes functionalized with azido-RB by CuAAC over the course of two minute total reaction time.

Kinetics of Copper-Free and Copper Catalyzed Cycloaddition in Solution

In order to gain insight into copper-free cycloadditions on a polymer brush, the kinetics of the solution reaction must first be investigated. The absorbances of DIBO and ADIBO in solution decrease with time at 315 nm and 326 nm, respectively, upon the

addition of azide. The alkyne/azide cycloaddition reaction is a second order reaction, dependent on the concentration of both azide and alkyne. The second order rate constants for copper free cycloadditions of DIBO and ADIBO with benzyl azide (25mM) in solution (Figure 4.8) are 0.07 $M^{-1}s^{-1}$ and 0.40 $M^{-1}s^{-1}$, respectively, both with a correlation coefficient (R^2) of 0.999. The faster rate of ADIBO over DIBO is due to the polarized alkyne bond generated by the nitrogen in the cyclooctyne ring, which increases distortion energy of ADIBO and further lowers activation energy of the cycloaddition reaction.^{28,29,40}



Figure 4.8. Solution kinetic plot of copper-free click reactions of DIBO and ADIBO with benzyl azide. Plots represent the disappearance of DIBO and ADIBO absorbances at 326 and 315, respectively.

While DIBO and ADIBO have a distinct absorbance band that can be observed, the CuAAC with PPG does not have a characteristic absorbance observed in solution. Furthermore, it is difficult to track the formation of the triazole spectroscopically because of its overall weak, broad absorbance at 330 nm. Therefore, CuAAC kinetics were used from a solution polymeric CuAAC reaction from the literature.⁵⁹ In the CuAAC reaction, the Matyjaszewski group used tridentate amine ligands, such as PMDETA, to stabilize the Cu(I) state and prevent oxidation to Cu(II), leading to a rate acceleration of CuAAC up to 250 times over reactions without the stabilizing ligand, yielding a second order rate constant of 0.032 M⁻¹s^{-1.59}

Kinetics of Alkyne Polymer Brush Functionalization

The absorbances as a function of time were measured for DIBO, ADIBO, and PPG functionalized brushes at λ_{max} for azido-RB covalently attached to the brush scaffold at 575, 571, and 577 nm, respectively (Figure 4.9). On the surface, one can assume pseudo-first order conditions for alkyne/azide cycloadditions due to the large excess of azido-RB in solution (40 mM) relative to the nanomolar concentrations of alkyne immobilized on the surface. The pseudo-first order rate equation is listed below in Equation 4.3:

$$A_t = A_0 e^{k't}$$
 (Equation 4.3)

where A_0 is absorbance at time zero, A_t is absorbance at time t, and k' is the pseudo-first order rate constant. Equation 4.3, however, does not accurately describe the reaction conditions of polymer brush functionalization. Functionalization of the brush is limited by either the number of alkyne sites on the polymer brush, or by limited access to alkyne sites on the polymer chains by diffusion, giving a final absolute absorbance value. This is represented graphically in Figure 4.10.



Figure 4.9. Absorbance data of functionalization time for DIBO (purple squares), ADIBO (green triangles), and alkyne (blue diamonds) with azido-RB. The graph illustrates absorbance changes until final absorbance A_{∞} is reached. The solid lines are meant to guide the eye.



Figure 4.10. Graphic representation of polymer brush functionalization when a functional pendant group (small green spheres) is placed in a solution with an absorbing reactive species (large red spheres) where L is the brush thickness. The functionalization occurs until a maximum amount of reactive species is incorporated into the brush within reaction time of t_{∞} .

In order to determine the pseudo-first order rate, the rate of reaction must be modeled as the change in absorbance relative to the total possible change in absorbance for the polymer brush. This model is called the pseudo-first order limited rate equation (Equation 4.4):

$$k't = \ln\left(\frac{A_o - A_{\infty}}{A_t - A_{\infty}}\right)$$
(Equation 4.4)

where k' is the pseudo-first order rate constant, and A_o , A_t , and A_∞ are the absorbance values at λ_{max} initially, at time interval t, and at the end of functionalization, respectively. The absorbance data at λ_{max} for azido-RB functionalization of the brushes was fit to Equation 4.4 and is shown below in Figure 4.11.



Figure 4.11. Pseudo-first order limited log plot of azide functionalization of polymer brushes containing reactive alkynes DIBO (pink hexagons), ADIBO (green circles) and alkyne (blue triangles).

The rate of azide functionalization of DIBO polymer brushes is the slowest at 7.7 x 10^{-4} s⁻¹ with a standard deviation between trials of 1.21 x 10^{-4} s⁻¹. The log plot of the rate fits well with the pseudo-first order limited model with a correlation coefficient (R²)

of 0.993. The rate of ADIBO polymer brush functionalization was faster at 4.4 x 10^{-3} s⁻¹ with a standard deviation between trials of 8.12 x 10^{-4} s⁻¹ and an R² value of 0.995. The rate of azide functionalization of the propargyl polymer brushes using a copper(I) catalyst was 2.0 x 10^{-2} s⁻¹ with a standard deviation between trials of 6.01 x 10^{-4} s⁻¹ and a correlation coefficient (R^2) of 0.950. This rate is much faster than either of the catalyst free polymer brush functionalizations. The overall ratio of the rates of polymer brush functionalization was 28.5:6.2:1 for CuAAC, ADIBO, and DIBO functionalized brushes, respectively. The pseudo-first order rate constants for DIBO and ADIBO in solution were calculated from the second order rates as $1.7 \times 10^{-3} \text{ s}^{-1}$ and $9.9 \times 10^{-3} \text{ s}^{-1}$, respectively, in order to contrast relative reaction rates from solution to the polymer brush functionalization rate. To directly compare the pseudo-first order rates of alkyne/azide coupling in solution and on the surface, the relationship of these rates is represented by the ratio of the pseudo-first order brush functionalization rate (k'_B) computed from Equation 4.4 to the pseudo-first order solution functionalization rate (k's) as the term alpha (α) in Equation 4.5. The relative rate of click reaction for all surface and solution kinetics is summarized in Table 4.5.

$$\alpha = \frac{k'_B}{k'_S}$$
 (Equation 4.5)

Table 4.5. Average pseudo-first order rates of azide functionalization of dibenzocyclooctynes (DIBO and ADIBO) and alkynes (PPG) studied on polymer brushes and in solution.

	Polymer Brush	Solution	α
	Rate (s^{-1})	Rate (s^{-1})	
DIBO	7.7 x 10 ⁻⁴	1.7 x 10 ⁻³	0.45
ADIBO	4.4 x 10 ⁻³	9.9 x 10 ⁻³	0.44
PPG	2.0 x 10 ⁻²	*3.2 x 10 ⁻²	0.63

*Solution CuAAC rate was used from a similar experiment in the literature.⁵⁹

A 55% decrease in reaction rate was observed for polymer brush functionalization with both of the cyclooctynes, DIBO and ADIBO, relative to the rate of azide cycloaddition in solution. The surface functionalization rate by CuAAC click was 63% of the overall solution rate. Sodium ascorbate, in addition to the PMDETA ligand, was used in the surface CuAAC reaction to keep the concentration of copper(I) sufficiently high. This additional measure was intended to prevent the copper redox rate from impacting the rate of polymer brush formation. Use of the CuBr/PMDETA/sodium ascorbate CuAAC system was previously used to crosslink insoluble polymer networks with a reaction time of less than 5 minutes, although no exact rate of reaction was determined.⁵ The overall ratio of solution reaction rates for ADIBO and DIBO were 5.8:1 (Figure 4.8). In comparing that ratio to the 6.2:1 that was observed on the surface,

the relative rates of ADIBO:DIBO on the surface and in solution are analogous, demonstrating no significant change in the distortion energy of the cycloalkyne of copper-free click chemistry when attached to the polymer brush backbone. Values of CuAAC relative to copper-free click are on the same order of magnitude in solution and on a surface: CuAAC:DIBO is 28.5:1 on the surface and 15.8:1 in solution. The agreement between relative rates of different alkyne/azide coupling reactions in solution (ADIBO:DIBO and PPG⁵⁹:DIBO) with the corresponding polymer brush functionalizations demonstrate that the confined brush environment has no significant impact on different cycloaddition mechanisms at the surface.

Analysis of Copper-Free Click Chemistry of ADIBO Polymer Brushes using Dilute Solution Azides

The pseudo-first order rates of azide functionalization for DIBO, ADIBO, and PPG derivatized brushes discussed in this study were conducted using a concentrated solution (40 mM) of azido-RB. In addition to using the high concentration of azide to assume pseudo-first order reaction rates with the polymer brush, the concentration was used to limit the possibility of small molecule mass transport within the brush layer, which can affect the rate of reaction. The kinetic experiment was repeated using similar ADIBO brushes, with a dilute azide solution (500 μ M) for functionalization. In this experiment, the overall concentration of azide in solution is still much greater than the potential concentration of active sites on the surface, approximately 25 nmol/cm²,⁴² which maintain pseudo-first order reaction conditions within the polymer brush. The solution azide will first functionalize the alkynes on the outer portions of the polymer brush (Figure 4.10), then undergo diffusion into the extended polymer brush chains,
functionalizing the buried alkynes as they penetrate into the brush. As polymer brush functionalization adds molar mass to the side chains, the polymer brush will begin to extend further away from the surface to accommodate the increased polymer density, increasing film thickness (L). Azides must diffuse into the brush matrix in order to functionalize buried alkyne sites closest to the substrate surface, which can cause a diminished reaction rate. The azide functionalization rate of the ADIBO brush is pseudofirst order, but the overall low number of azide molecules in solution will now have to diffuse into the 50 nm brush matrix. The kinetic experiment was repeated with the dilute solution azide and the absorbance at $\lambda_{max} = 571$ nm versus time was recorded and is shown in Figure 4.12.



Figure 4.12. Increase in azido-RB absorbance with time for a dilute (500 μ M) azide solution. The light purple line is meant to guide the eye.

Absorbance of the azido-RB dye on the brush increases steadily until 1800 seconds, where the absorbance increase begins to taper and eventually plateaus, reaching A_{∞} at 16000 seconds. Fit of all data (from 0 to 16000 seconds) to the pseudo-first order limited equation yields a rate constant of 3.28 x 10⁻⁴ s⁻¹ with an 85% confidence interval, indicating a poor fit.



Figure 4.13. Kinetic fit of dilute azide/ADIBO brush functionalization. The red and blue lines represent two regimes of brush functionalization: pseudo-first order limited regime from 0-2300 seconds (blue triangles) and a pseudo-first order diffusion limited regime from 2301-16000 seconds (red diamonds) using the diffusion parameter, a.

Using a pseudo-first order limited model does not fit the data for low concentration azide functionalization, as shown by the two distinct slopes in Figure 4.13. The rate is reduced after many of the outermost alkyne sites have been functionalized, but

appears to remain consistent after that point. To model an accurate representation of the rate, one must examine the reaction rate in a regime of strong diffusional resistance⁶⁰ where reaction conversion decreases with time.

$$\begin{cases} -r'_{A} = k'[A]a \\ -\frac{da}{dt} = k_{d}a = C \end{cases}$$
 (Equation 4.6)

As stated in Equation 4.6, the rate of azido-RB functionalization (r'_A) is the product of the pseudo-first order rate constant (k'), the concentration of azide ([A]), and a deactivation term (a), which accounts for the change in reaction kinetics by the rate of diffusion, k_d . The assumption is made that the diffusion rate does not change with time and is a constant (C), which is plausible upon examination of the data in Figure 4.13. If the data is split into two regimes, the first from 0 to 2300 seconds, and the second from 2301 to 16000 seconds, it is observed that the first regime is pseudo-first order limited with k' = 8.01 x 10^{-4} s⁻¹ with a R² value of 0.994, and the second regime is diffusion limited and linear when graphed as a pseudo-first order limited reaction with a value of k' = 1.87 x 10^{-4} s⁻¹ and R² value of 0.980. There is diffusion into the polymer brush that retards the reaction rate, but at a constant factor, da/dt = C. The ratio of the pseudo-first order limited rate constants for the two regimes yields the deactivation term, a = 0.233, which indicates a three-quarters decrease in reaction rate once the brush functionalization becomes diffusion limited. The derived diffusion limited pseudo-first order rate equation, including the deactivation constant, a, is shown in Equation 4.7.

$$k't = a \ln \left(\frac{A_o - A_\infty}{A_t - A_\infty} \right)$$

$$a = \frac{A_\infty}{A'_o - A_\infty}$$
(Equation 4.7)

The term A_0 ' is the absorbance value where the brush functionalization enters a diffusion limited regime and the slope of the first order limited equation begins to deviate. In order to fully evaluate diffusion limited kinetics, however, further concentration studies of polymer brush functionalization need to occur using a variety of dilute solutions and brushes of varying thicknesses. These experiments will determine when the diffusion limited regime begins for small molecule polymer brush functionalization.

Conclusions

The utility of using alkyne functional polymer brushes rests on knowing the optimum conditions and limitations on dense polymer brush functionalization. It has been demonstrated that immobilized alkynes of differing rates and structures can covalently couple azides on a 50 nm polymer brush surface within an hour of reaction time. The time-dependent functionalization of alkynes is not as quantitative as has been demonstrated with activated esters and primary amines, but still offers a straightforward route to densely packed polymer scaffolds with direct azide functionalization. Click kinetics for both catalyst free cycloaddition and conventional CuAAC demonstrate pseudo-first order limited rates that are 40 to 60% slower within a polymer brush than corresponding reactions in solution. The relative rates of reaction of DIBO, ADIBO, and PPG in solution agree well with the difference in reaction rates between the same functional groups on the polymer. The similarity in relative rate between CuAAC and copper-free click chemistry in solution and on a surface demonstrates the negligible effect of a densely packed polymer brush scaffold on the mechanism and geometry of the reaction, leaving possibilities open to generate new functional polymer brush surfaces with a variety of derivatizable pendant groups. For small molecule azides, mass transport does not influence the kinetic rate of a 50 nm functionalized polymer brush as long as the azide concentration is sufficiently high. Preliminary work on low concentrations of azides indicates that for a thin (50 nm) polymer brush, diffusion limited reaction slows the reaction rate to one-quarter of the non-diffusion limited rate.

Development of a tunable alkyne functional polymer brush scaffold from an active ester polymer brush provides alternative chemistry of using azides instead of amines for densely packed post-polymerization modification at the surface. Alkyne/azide cycloaddition is amenable to fabrication of functionalizable polymer scaffolds due to their ease of derivatization and increased tolerance to different reaction conditions. The non-competitive nature of alkyne/azide cycloaddition and aminolysis makes using functional polymer brushes a well-controlled system to develop new polymer interfaces, where both types of pendant functionality can be incorporated into a well-defined polymer scaffold.

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CHAPTER 5

SPATIALLY-RESOLVED MULTIFUNCTIONAL POLYMER BRUSHES THROUGH ORTHOGONAL SELF-ASSEMBLY¹

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Abstract

In this chapter, the orthogonal deposition of catechol-functionalized initiators selectively on titanium oxide in the presence of silicon oxide for the selective and controlled surface initiated polymerization of functional monomers. nhydroxysuccinimide 4-vinyl benzoate (NHS4VB) and 4-(trimethylsilyl) ethynylstryene (TMS styrene) is described. Exclusive polymer growth on the titanium oxide was confirmed by measuring the change in film thickness, the static contact angle, infrared spectroscopy, and scanning electron microscopy. Dual polymer brush scaffolds containing spatially-resolved regions of both poly(NHS4VB) and poly(TMS styrene) were fabricated by selective deposition of a second initiator and subsequent polymerization using a different polymerization technique. Functionalization of the active ester by aminolysis and the alkyne by copper catalyzed cycloaddition with aminomethylpyrene and azido-Rhodamine B conjugate, respectively, demonstrated the spatial fidelity of the patterned polymer brushes by fluorescence microscopy.

Introduction

Specialized surfaces with high resolution patterns of different functionality can be useful in the development of nanoscale biological mimics, bioarray technology, and the electronics industry. Spatially resolved functionality on polymer surfaces has been previously demonstrated by "top down" patterning methods such as stamping or photolithography.¹⁻⁴ Recent thin film deposition techniques such as glancing angle deposition (GLAD) can fabricate 3D nanostructured surfaces on the order of nanometers.⁵⁻⁷ GLAD is compatible with several types of materials including metals and metal oxides. Other specialized structures such as nanocables and nanowires have been fabricated that contain multilayers of different materials as well.⁸ The selective deposition of different materials to form these surfaces are beyond the current resolution limits of lithographic methods and pose a further challenge in patterning chemical functionality on a 3D structure. In order to customize polymer interfaces on the nanoscale, spatially-resolved surface chemistry must be controlled from a "bottom up" method.

Selective chemical adsorption onto two or more substrates from a single solution containing one or more reactive species is known as orthogonal self-assembly. This orthogonal surface functionality from the "bottom up" has been previously demonstrated in the formation of selective self-assembled monolayers on different metal and oxide surfaces. The first examples of orthogonal surface functionalization were using gold-thiol and alumina-carboxylic acid chemistry to form mutually exclusive self-assembled monolayers (SAM).⁹ Other examples of orthogonal monolayers include monolayer formation on gold/SiO₂ surfaces using thiol and silane monolayers,^{10,11} and selective

functionalization of isonitrile and thiol monolayers on platinum and gold surfaces.^{12,13} Selective formation of adsorbed monolayers was also conducted on TiO_2 and SiO_2 using phosphonic acid groups, which permanently bind to TiO_2 , while the weak Si-O-P bond on silicon oxide is easily removed by hydrolysis and backfilled with a silane SAM.¹⁴

Recent biological inspiration for novel orthogonal self-assembly originates from the attachment of mussels to solid surfaces in turbulent water environments. Mussels secrete a byssus, or tether filament, that strongly adheres itself to the surface, which is characterized as a strong, highly extensive, hard cuticle coat with robust mechanical properties.¹⁵ The main component of the byssus, and the source of its mechanical strength, is an iron – catechol complex, which creates collagen – like peptides.¹⁵ Catechols have been previously studied in the literature to bind strongly to a variety of metal oxide surfaces.¹⁶⁻¹⁹ Prior research on catechol macromolecules on surfaces has focused on the covalent attachment of antifouling polymers such as poly(ethylene glycol) (PEG) to the surface in a "grafting to" method.²⁰⁻²³ Catechol-oxide surfaces have also been used to form nanoscale field-effect transistors²⁴ and chemical gradients on the surface.²⁵ Catechols have an especially strong chelating effect with titanium(IV) oxide²⁶⁻ ²⁸ where both oxygens of the catechol coordinate to an individual titanium atom, forming a bidentate complex.^{16,24,29-32} Specifically, it has been calculated that a Ti=O defect site on the titanium(IV) oxide surface catalyzes the formation of the bidentate structure with an adsorption energy of about 25-30 kcal/mol.^{33,34} The functionalization of Ti(IV) oxide with catechol is pH dependent, forming the most metal-ligand complexation when pH is neutral (pH 6 to 8).^{29,35} The Zürcher group demonstrated selectivity of catechol functionalization by grafting catechol-PEG chains to silicon, titanium, and niobium

oxides by careful control of pH. They concluded that the optimal attachment of catechol on a surface occurred when the pH of the solution was equal to the isoelectric point (IP) of the metal oxide.³⁶ Selective catechol functionalization between two metal oxides of differing IPs will permit orthogonal self-assembly of robust catechol-functionalized SAMs.

Catechol-initiators have been synthesized for controlled surface initiated polymerization by atom transfer radical polymerization (ATRP)^{37,38} and ring-opening metathesis polymerization (ROMP)³⁹ to make polymer brush-coated surfaces. Polymer brushes have been previously demonstrated to form customizable interfaces from the bottom up, using a functional pendant group such as an n-hydroxysuccinimide (NHS) ester for aminolysis to attached solution amines along the 3D stretched polymer backbone.^{40,41} Polymers have also been synthesized with an alkyne or azide functional pendant group for copper-catalyzed azide alkyne cycloaddition (CuAAC),⁴²⁻⁵⁰ known as one of the classic examples of "click" chemistry.⁵¹ The combination of the aminolysis of an activated NHS ester and the CuAAC of an alkyne with an azide species together allow for orthogonal approaches to post-polymerization modification, as has been demonstrated in solution polymer modification,⁵² on microgel particles⁵³ and on chemical vapor deposited polymer surfaces.⁴

In this work, the selectivity of monolayer formation of catechol initiators on titanium (IV) oxide over silicon dioxide and surface initiated polymerization of functional brush scaffolds from TiO_2 will be demonstrated. Furthermore, optimization of reactions conditions will occur to selectively polymerize a second functional monomer on the remaining SiO_2 sites remaining by a different controlled polymerization technique.

Dual functional polymer templates will be formed by the combination of orthogonal surface functionalization using the catechols and orthogonal post-polymerization modification of brushes by aminolysis and CuAAC. These experiments will form a protocol to develop a spatially-resolved dual polymeric scaffold, which affords densely packed and customizable functionalization from the bottom up.

Experimental

Materials

All solvents, with the exception of tetrahydrofuran (THF), toluene, and dichloromethane (DCM) were purchased from EMD (ACS grade) and used as received. THF was dried by sodium-ketyl distillation and toluene and DCM were purified and dried by distillation over calcium hydride. Anhydrous dimethylsulfoxide (DMSO) and dimethylformamide (DMF) (Drisolv, 99.8% by GC) were purchased from EMD. Silicon wafers (orientation <100>, native oxide) were purchased from University Wafer. TiO₂ target for physical vapor deposition was purchased from Kurt J. Lesker. N,N',N, N', N'' pentamethyldiethylenetriamine (PMDETA) was purchased from Alfa Aesar and distilled prior to use. All other chemicals were purchased from Sigma Aldrich and used as received.

Fabrication of TiO₂ and SiO₂ Patterned Substrates

Stripes of 1 cm wide TiO_2 were deposited onto clean silicon wafers (University Wafer) by physical vapor deposition from a TiO_2 target using magnetron sputtering (PVD75, Kurt J. Lesker). A tape mask was used to control stripe width. Wafers were rinsed with isopropanol and blown dry before and after deposition. The titanium(IV)

oxide was deposited at 0.5 Å/s using a 50 W power setting and a 5 mTorr capman pressure.

Initiator Synthesis

Synthesis of 2-Bromo-N-(3,4-dihydroxyphenethyl)-2-methylpropanamide (ATRP Catechol)

The synthesis of the catechol ATRP initiator was an adaptation from two procedures in the literature.^{36,37} A precursor was first synthesized: 2,5-dioxocyclopentyl 2-bromo-2-methylpropanoate. In a round bottom flask under nitrogen atmosphere with 30 mL of dry THF, pyridine (2.7 mL, 33.6mmol) and n-hydroxysuccinimide (3.52 g, 30.6 mmol) were added and stirred vigorously. Bromoisobutyryl bromide (5.0 mL, 30.6 mmol) in 5 mL dry THF were added to the solution dropwise and allowed to stir overnight. The white precipitate was removed by filtration and the solution was reduced by rotary evaporation to remove the solvent. The product was extracted three times with ethyl acetate and brine, dried over magnesium sulfate and the solvent was removed by rotary evaporation. The product was recrystallized using isopropanol to yield white crystals. 7.2 g, 90% yield. ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 2.86 (s, 4H, CH₂); 2.08 (s, 6H, CH_3). Next, dopamine HCl (0.716 g, 3.78 mmol) and n-methyl morpholine (0.663 g, 6.55 mmol) were dissolved in 70 mL of ethanol under nitrogen atmosphere. 2,5-dioxocyclopentyl 2-bromo-2-methylpropanoate (1.0 g, 3.78 mmol) was dissolved in 5 mL chloroform and added to the ethanol solution dropwise. The solution was stirred vigorously for 2.5 hours. Most of the ethanol was removed under reduced pressure to concentrate the solution to approximately 20 mL. 100 mL of ether was then added and the solution was put in the freezer (4 °C) overnight. White precipitate of N-

methylmorpholine oxide was removed by filtration and the ether solution was evaporated to dryness. The product was purified by flash chromatography (2:1 ethyl acetate/ hexane) where the first fraction to elute was the desired product. 0.7 g, 61.4% yield. ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 6.89 (br s, 2H, OH); 6.80 (d, 8.1 Hz, 1H, Arom.); 6.74 (d, 2 Hz, 1H, Arom.); 6.60 (dd, 8.6 Hz, 1H, Arom); 6.53 (br s, 1H, NH); 3.46 (q, 6.8 Hz, 2H, CH₂NH); 2.71 (t, 7 Hz, 2H, CH₂CH₂NH); 1.91 (s, 6H, CH₃).

Synthesis of N-(3,4-dihydroxyphenethyl)undec-10-enamide (ROMP Catechol)

The synthesis of the catechol ROMP initiator was an adaptation from two procedures in the literature.^{36,37} A precursor, n-hydroxysuccinimide undec-10-enotate, was first synthesized. In a round bottom flask under nitrogen atmosphere with 30 mL of dry THF, pyridine (2.0 g, 25.6 mmol) and n-hydroxysuccinimide (2.68 g, 23.3 mmol) were added and stirred vigorously. 10-undecenoyl chloride (5.0 mL, 23.3 mmol) in in 5 mL dry THF were added to the solution dropwise and allowed to stir overnight. The white precipitate was removed by filtration and the solution was reduced by rotary evaporation to remove the solvent. The product was extracted three times with ethyl acetate and brine, dried over magnesium sulfate and the solvent removed by rotary evaporation.

Dopamine HCl (2.76 g, 14.57 mmol) and n-methyl morpholine (2.55 g, 25.2 mmol) were dissolved in 100 mL of ethanol under nitrogen atmosphere. N-hydroxysuccinimide undec-10-enoate was dissolved in chloroform (10 mL) and added to the ethanol solution dropwise. The reaction stirred overnight. Ethanol was removed under reduced pressure and reaction was extracted three times with ethyl acetate and brine, dried over magnesium sulfate and evaporated to dryness. The crude product was

purified by flash chromatography (2:1 ethyl acetate/ hexane) and was the second spot to elute. 0.7 g, 60% yield. ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 7.8 (br s, 2H, OH); 6.81 (d, 8.7 Hz, 1H, Arom.); 6.74 (d, 2.4 Hz, 1H, Arom.); 6.56 (dd, 7.6 Hz, 1H, Arom); 6.53 (br s, 1H, NH); 5.73 (m, 1H, CH=CH₂); 5.00 (d, 2.2 Hz, 1H, CH=CH₂); 4.90 (d, 8.6 Hz, 1H, CH=CH₂); 3.46 (q, 6.8 Hz, 2H, CH₂NH); 2.68 (t, 8.1 Hz, 2H, CH₂CH₂NH); 2.15 (t, 8.9 Hz, 2H, CH₂-C=O); 2.05 (q, 7.3 Hz, 2H, CH₂CH=CH₂); 1.58 (m, 2H, CH₂CH₂C=O); 1.25 (m, 10H, CH₂).

Synthesis of 4,4'-(Diazene-1,2-diyl)bis(4-cyano-N-(3,4-dihydroxyphenethyl)pentanamide) (AIBN-Catechol)

4,4-Azobis(4-cyanovaleric acid) (2.19 g, 7.8 mmol) was added to thionyl chloride (50 mL) under nitrogen atmosphere. The reaction mixture was refluxed for 15 min in a 100 °C oil bath. The hot solution was immersed in an ice bath and cooled to room temperature. Excess thionyl chloride was removed by vacuum evaporation at room temperature to yield a yellow solid. To the crude product was added 25 mL of dry tetrahydrofuran and the solution was bubbled with argon gas one hour to remove dissolved oxygen. In a separate flask, sodium borate decahydrate (2.97 g, 7.8 mmol) was dissolved in 18 M Ω nanopure water (40mL) and also deoxygenated with argon gas for one hour. Dopamine HCl (2.975 g, 15.7 mmol) was added to the water solution followed by sodium carbonate (2.66 g, 25.11 mmol) and dissolved. The aqueous solution was kept under inert atmosphere and chilled to 0°C in an ice bath and stirred vigorously. The THF/cyanovaleric acid chloride solution was added dropwise to the chilled solution and stirred 24 hours. The reaction mixture was acidified to pH 2 using 2M HCl solution and washed three times with 30 mL portions of ethyl acetate. The ethyl acetate layers were collected, dried with MgSO₄, and evaporated to dryness. The crude product was purified

by column chromatography using ethyl acetate/hexane (2:1) as the eluent. The product was a yellow solid. 0.923g, 25.6% yield. ¹H NMR (DMSO D₆, 300 MHz) δ (ppm): 8.02 (br s, 1H, OH); 8.00 (br s, 1H, OH); 6.61 (d, 7.9 Hz, 2H, Arom.); 6.57 (s, 2H, Arom.); 6.44 (d, 8.0 Hz, 2H, Arom.); 6.34 (br s, 1H, N*H*); 3.18 (d, 5.9 Hz, 4H, C*H*₂NH); 2.54 (d, 7.6 Hz, 4H, C*H*₂CH₂NH); 2.38-2.24 (m, 8H, C*H*₂); 1.68 (s, 3H, C*H*₃); 1.64 (s, 3H, C*H*₃). ¹³C NMR (DMSO D₆, 300 MHz) δ (ppm): 173.29, 145.70, 144.16, 130.81, 119.84, 116.58, 116.11, 72.62, 35.23, 33.94, 32.69, 30.71, 23.60, 21.42.

Synthesis of 2,2,6,6-Tetramethyl-1-(1-(4-(((11-

(*trichlorosilyl*)*undecyl*)*oxy*)*methyl*)*phenyl*)*ethoxy*)*piperidine* (*Chlorosilane-NMP*) was synthesized according to literature procedures.^{54,55}

Synthesis of 2,2,6,6-Tetramethyl-1-(1-(4-(((11-

(trimethoxysilyl)undecyl)oxy)methyl)phenyl)ethoxy)piperidine (Methoxysilane-NMP)

The Chlorosilane-NMP was converted to the methoxy analog by addition dropwise into methanol until the solution stopped fuming. The methanol was removed by rotary evaporation and the methoxysilane product was used as is.

Deposition of Monolayers

Orthogonal SAM of Catechol ATRP on Dual TiO₂/SiO₂ surfaces

1. Selective Catechol Deposition on TiO_2 Substrates were cleaned by rinsing the surfaces with isopropanol, then water and drying under a stream of nitrogen. The surface was argon plasma cleaned for 2 minutes (0.8 mbar), then ozone cleaned for 5 minutes to make the surface completely hydrophilic. The substrates were covered in a 1 mg/mL solution of the catechol initiator in water and placed in the dark for 24 hours. The substrates were then rinsed with methanol and water and dried under nitrogen stream.

2. Selective Catechol Deposition on SiO_2 The substrates were covered in a 1 mg/mL solution of the catechol initiator in water/ HCl solution (pH 2) and placed in the dark for 24 hours. The substrates were then rinsed with methanol and water and dried under nitrogen stream.

Orthogonal SAM of Catechol ROMP on Dual TiO₂/SiO₂ surfaces

Substrates were cleaned by rinsing the surfaces with isopropanol, water, and drying under a stream of nitrogen. The surface was argon plasma cleaned for 2 minutes (0.8 mbar), then ozone cleaned for 5 minutes to make the surface completely hydrophilic. The substrates were covered in a 1 mg/mL solution of the catechol initiator in a 5:4:1 (v/v) solution of water, methanol, and ethyl acetate and placed in the dark for 24 hours. The substrates were then rinsed with ethyl acetate, methanol, and water and dried under nitrogen stream.

Orthogonal SAM of Catechol AIBN on Dual TiO₂/SiO₂ surfaces

Substrates were cleaned by rinsing the surfaces with isopropanol, then water and drying under a stream of nitrogen. The surface was argon plasma cleaned for 2 minutes (0.8 mbar), then ozone cleaned for 5 minutes to make the surface completely hydrophilic. The substrates were covered in a 1 mg/mL solution of the catechol initiator in methanol and placed in the dark for 24 hours. The substrates were then rinsed with methanol and water and dried under nitrogen stream.

Silane SAM Formation of NMP Initiator on SiO₂

Chloro- and methoxy- silane NMP initiators were both deposited on silicon oxide surfaces by the same procedure. Substrates from prior catechol monolayer or polymerization reactions, and all dry, degassed reagents were transferred into a nitrogen filled glovebox. One drop of initiator was mixed with 20 mL dry, degassed toluene (approximate concentration 10 mM) and the solution was filtered through a 0.45 μ m PTFE filter and poured over the clean silicon wafers in a glass staining jar, sealed and brought outside the glovebox. After varied reaction times (15 minute intervals up to 1 hour for chlorosilanes, 30 minutes for methoxysilane) the substrates were removed, rinsed with freshly distilled toluene, and dried under a stream of nitrogen.

Monomer Synthesis

4-(*Trimethylsilyl*) ethynylstryene (TMS styrene)⁵² and 2,5-Dioxocyclopentyl bicyclo[2.2.1]hept-5-ene-2-carboxylate (Norbornene- NHS ester or Nb-NHS)

TMS styrene and Nb-NHS were synthesized according to literature procedures.⁵⁶ Synthesis of N-hydroxysuccinimide 4-Vinyl Benzoate (NHS4VB)

NHS4VB was prepared in a three step procedure from 4-bromobenzaldehyde. Briefly, 4-bromobenzaldehyde was converted to 4-bromostyrene using Wittig chemistry with triphenylphosphine methyl ylide.⁵⁷ 4-Bromostyrene was converted to 4-vinylbenzoic acid through Grignard formation and quenching with CO₂. Finally, coupling of nhydroxysuccinimide with 4-vinylbenzoic acid gave the active ester NHS4VB.^{58,59}

Polymerization Methods

Atom Transfer Radical Polymerization: NHS4VB, TMS styrene, and NIPAM

The initiator substrate was placed in a dry, flat bottom Schlenk flask in the glovebox. The NHS4VB monomer (1.65 g, 6.75 mmol) and 1.2 mL DMSO were added to the Schlenk flask. Separately, a stock solution was made that consisted of 0.5 mL DMSO, N,N,N',N'',Pentamethyldiethylenetriamine (PMDETA, 423 µL, 2.03 mmol), copper (I) bromide (39 mg, 0.27 mmol) and copper(II) chloride (7.26 mg, 0.05 mmol).

An aliquot of 230 μ L of the stock solution was added to the Schlenk flask, which was then sealed, brought outside the glovebox and stirred in a 50 °C oil bath for 1.5 hours to achieve a polymer brush of approximately 50 nm thickness. The flask was then opened and exposed to air, and the wafers were rinsed vigorously with DMF and dried under a stream of argon. The procedure for ATRP of TMS styrene is identical, using 0.45 g (2.25 mmol) of TMS styrene monomer. ATRP of n-isopropylacrylamide (NIPAM) was carried out according to literature procedures.⁶⁰ To remove alkyl halide initiator chain ends *in situ*, 50 μ L of degassed tributyl tin hydride was added to the polymerization before it was exposed to oxygen and polymerization continued for an additional 10 minutes before the reaction was opened to air and the substrates were rinsed.

Nitroxide Mediated Radical Polymerization of 4-(Trimethylsilyl) Ethynylstryene (TMS styrene)

The initiator substrate was placed in a flat-bottom Schlenk flask under argon atmosphere. 1 mL of a 25 weight percent solution of TMS styrene in anhydrous anisole was added to the flask and bubbled with argon for 1 hour. The flask was sealed and placed in a 125°C oil bath overnight. The substrate was removed, rinsed with THF, then soxhlet extracted with THF at 80°C for at least 2 hours to remove any physisorbed polymer. The substrate was dried under a stream of nitrogen.

Ring Opening Metathesis Polymerization of Nb-NHS

Substrates and all dry, degassed chemicals were transferred to the glovebox. Initiator substrates were heated at 40°C in a solution of Grubbs generation 2 catalyst (22 mg, 0.026 mmol) in 1 mL DCM for 75 minutes, then rinsed thoroughly with fresh DCM and transferred to a solution of Nb-NHS in DCM (100 mg/mL) in a scintillation vial and sealed. The polymerization occurred for 1 hour, and was quenched with ethyl vinyl ether to terminate polymerization. The substrate was removed from the glovebox and rinsed with DCM and DMF and dried under nitrogen.

Free-Radical Polymerization of NIPAM

An 80 weight percent solution of NIPAM in dichloromethane containing the catechol AIBN substrate was degassed by several freeze-pump-thaw cycles in a flask which was sealed and subjected to UV light (350 nm) for at least 16 hours. The substrate was removed, and placed in a soxhlet extractor with THF at 80°C overnight to remove physisorbed PNIPAM from the surface. The substrate was then removed, rinsed with THF and dried under nitrogen.

Synthesis 4-(N-3-azidopropyl)sulfonylamido Lissamine Rhodamine B (azido-RB)

3-Azidopropyl amine (0.049 g, 0.49 mmol) was added to a solution of Lissamine Rhodamine B sulfonyl chloride (0.200 g, 0.347 mmol) in DMF (3 mL) followed by N,Ndiisopropylethylamine (0.072 g, 0.628 mmol) and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated and the product was purified by chromatography (CHCl₃:MeOH 15:1) to provide dark red crystals.

Post-Polymerization Modification

Functionalization of Poly(NHS4VB) Brushes with Reactive Alkyne Primary Amines

Poly (NHS4VB) brushes were converted to functionalized amide derivatives of 4-vinyl benzoic acid using aminomethylpyrene (Py-N) (0.12 M in dry DMF) at 40 °C for two hours with triethylamine as a proton acceptor.⁴¹

CuAAC of Poly(ethynyl styrene) and Azido RB

Poly(TMS styrene) was first deprotected to form poly(ethynyl styrene) by soaking the substrate in 6M potassium hydroxide in methanol for 3 hours. The substrate was rinsed well with methanol, water, and then dried under a stream of nitrogen. A flat bottom Schlenk flask was put under argon blanket, where a 1 mL solution of 40 mM azido RB in methanol was added and allowed to equilibrate. Separately, a stock solution of copper (I) bromide (28.7 mg, 0.2 mmol), PMDETA (41.8 μ L, 346.6 mg, 0.2 mmol), and sodium ascorbate (79.2 g, 0.4 mmol) was prepared in a 10 mL DMF/water solution (9:1, v/v) and bubbled with argon for 1 hour. A 100 μ L aliquot of the stock solution was added to the azido-RB solution and stirred to equilibrate the solution. Immediately, the stopper was removed from the Schlenk flask and a poly(ethynyl styrene) polymer brush was immersed in the solution and the Schlenk flask was closed and remained under an argon blanket at room temperature for 30 minutes. The substrate was removed, rinsed well with methanol, DMF, DCM, and dried under a stream of nitrogen.

Characterization

Spectroscopic ellipsometry was performed on a J. A. Woolham M-2000v spectroscopic ellipsometer with a white light source at 65°, 70° and 75° angles to the silicon wafer and delta (Δ) and psi (Ψ) were measured as a function of wavelength between 400 and 1000 nm. Film thicknesses were determined using a three and four layer models (Si, SiO₂, and polymer for SiO₂ surfaces and Si, SiO₂, TiO₂ and polymer for TiO₂ surfaces) using the Cauchy equation, which allowed for simultaneous modeling of film thickness and refractive index. The parameters a and b are also fitted with the Cauchy model, but have an average value of a = 1.44 and b = 0.013. The modeling software is integrated specific to the spectroscopic ellipsometer. Thickness measurements for all azido-RB functionalized brushes were also measured by profilometry (Veeco Dektak 150). Static contact angle measurements were taken on a Krüss DSA 100. UV-Vis spectroscopy was taken on a Varian 50Bio spectrometer. Atomic force microscopy images were taken using tapping mode on a Multimode NanoScope IIIa (Digital Instruments/Veeco Metrology) using silicon AFM probes with a 300 kHz resonant frequency and a 40 N/m spring constant. Scanning electron microscope measurements were taken on an EDAX Inspect F.FEG-SEM. Elemental analysis of the surface was conducted using energy-dispersive x-ray spectroscopy (EDS) attached to the SEM instrument and analyzed by Genesis integrated software. FTIR measurements were taken with a Nicolet Model 6700 with grazing angle attenuated total reflection accessory (GATR) at 256 scans with 4 cm⁻¹ resolution. Fluorescence microscopy pictures were taken using a Zeiss AX10 Observer AX10 inverted microscope with a X-cite Series 120 fluorescent light source and Chroma Technology filters: DAPI no. 49 (365 nm excitation, 445 and 450 nm emission) model GFP no. 38 filter (470 nm excitation, 525 emission).

Results and Discussion

Orthogonal Initiator Deposition on Titanium (IV) Oxide over Silicon Oxide Surfaces

Orthogonal SAM formation of three different catechol initiators was demonstrated on TiO_2 deposited on silicon wafers. The initiators studied were for atom transfer radical polymerization (ATRP), ring opening metathesis polymerization (ROMP) and free radical (FRP) polymerization. Representative catechol-functionalized initiators and following polymerization reactions are shown below in Figure 5.1.



Figure 5.1. Selective polymerizations on titanium oxide surfaces using catechol initiators to form polymer brushes of poly(NHS4VB) by ATRP (a), poly(Nb-NHS) by ROMP (b) and PNIPAM by FRP (c).

The isoelectric points (IP) of TiO_2 and SiO_2 are 6 and 2, respectively.³⁶ Catechol monolayer depositions for the three initiators were executed at pH 6 to selectively functionalize the neutral TiO_2 surface sites over the negatively charged Si-O⁻ sites.

Orthogonal deposition of the initiators was confirmed by contact angle and spectroscopic ellipsometry measurements listed in Table 5.1.

Table 5.1. Thickness and contact angle changes for selectively-functionalized TiO_2 metal oxides of catechol ATRP, ROMP, and FRP initiator monolayers. *Changes in surface thickness in addition to bare oxide layer.

	SiO ₂		Ti	O_2
	thickness	contact angle	thickness	contact angle
	(nm)	(degrees)	(nm)	(degrees)
Bare Substrates	2.2	0	9.7	0
*Catechol ATRP	0.9	0	2.1	66
*Catechol ROMP	0.0	4	3.1	86
* Catechol FRP	0.0	15	2.0	30

The silicon oxide surface remains very hydrophilic after monolayer deposition, completely wetting the surface for the catechol ATRP initiator, and having a maximum contact angle change of 3° and 15° for the ROMP and FRP catechol initiators. The SiO₂ surface has a negligible thickness change of less than 1 nm. The titanium oxide surface increases in film thickness by 2 nm upon catechol ATRP adsorption, 3 nm for catechol ROMP, and 2 nm for catechol FRP. There is a large increase in static contact angle for TiO₂ surfaces for ATRP, ROMP and FRP catechol initiators of 66° , 86° , and 30° , respectively. Determination of surface topography of the catechol monolayers on TiO₂ was imaged (Figure 5.2). The catechol functionalized TiO₂ surface (Figure 5.2b) was featureless and had an increase in root mean squared (RMS) roughness by only 0.2 nm from 0.402 nm to 0.614 nm from the bare oxide (Figure 5.2a).



Figure 5.2. AFM height images of bare TiO_2 (a) and TiO_2 surface functionalized with catechol ATRP initiator (b). RMS roughnesses of the surfaces are 0.402 nm and 0.614 nm, respectively.

The orthogonal catechol SAMs were then used to selectively initiate different polymerization mechanisms from the surface. Free radical initiation of the AIBN catechol monolayer demonstrated the photo-stability of the Ti-O-C bond as $1.2 \ \mu m$ of PNIPAM grew selectively on the TiO₂ side, and only 1 nm remained on the SiO₂ after Soxhlet extraction. Polymer brushes of PNIPAM, Poly(NHS4VB), and poly(TMS styrene) were selectively grown from catechol monolayers containing an alkyl halide ATRP initiator. PNIPAM ATRP brushes were first grown to demonstrate the exclusive growth of polymer brushes on TiO_2 from a commercially-available monomer. PNIPAM on TiO_2 had a thickness of 94 nm with only 0.67 nm of PNIPM on the SiO_2 side. PNIPAM thickness was comparable to ATRP of NIPAM on chlorosilane ATRP initiator substrates under identical reaction conditions. The contact angle difference between the two halves of the substrate containing different oxides is 20°. In addition to the thickness data, presence of the catechol monolayer and PNIPAM polymer exclusivity on TiO_2 was confirmed by GATR-FTIR, shown in Figure 5.3.



Figure 5.3. Selective SAM and surface initiated polymerization of NIPAM by ATRP on TiO_2 surfaces over SiO₂. Monolayer formation is shown on silicon oxide (a) titanium oxide (b) and PNIPAM brushes grown on SiO₂ (c) and TiO₂ (d).

The FTIR spectra were taken for each side of the substrate and demonstrate that catechol ATRP initiator is selective for TiO₂ by the C=O stretching at 1653 cm⁻¹ and N-H stretching at 3345 cm⁻¹, while the only peaks evident on the silicon oxide after initiator are a small amount of alkyl stretching from 2960-2850 cm⁻¹ and a small Si-O and Si-O-C stretching at 1050 and 1220 cm⁻¹, respectively. These absorbances are most likely attributed to small alkyl impurities on the silicon oxide after monolayer deposition, but do not show amide stretching indicative of the ATRP initiator. After polymerization, characteristic absorbances of PNIPAM are observed on TiO₂ only (amide I and II bands at 1643 and 1547 cm⁻¹ and CH₃ antisymmetric and symmetric deformations at 1449 and 1384 cm⁻¹). Chemical functionality on SiO₂ before and after NIPAM polymerization remains consistent, indicating no significant polymer grown from the silicon oxide surface.

ATRP from catechol initiators was further demonstrated in the ability to grow functional polymers from the surface and derivatize the brushes. Figures 5.3 and 5.4 show the formation of poly(TMS styrene) and poly(NHS4VB) brushes from catechol ATRP initiators with subsequent deprotection and functionalization of both with azido-Rhodamine B (azido-RB) and aminomethylpyrene (Py-N), respectively. A table of complete infrared assignments for Figures 5.3 through 5.5 is included in Table 5.2.

Surface	Frequency (cm ⁻¹)	Assignment [*]	Figure
Catechol ATRP	3445	N-H str	5.3b, 5.4a, 5.5a
initiator	2960, 2877	CH ₃ str	
	2922, 2856	CH ₂ str	
	1653	C=O str	
	1456	CH ₃ str	
	1224,1047	C-O str	
PNIPAM	3427	N-H str	5.3d
	2973, 2899	CH ₃ str	
	2932, 2871	CH ₂ str	
	1643	C=O str	
	1547	N-H str	
	1449, 1384	CH ₃ deformation	
	1367	C-N str	
Poly(TMS styrene)	2960, 2898	CH ₃ str	5.4b
	2926, 2855	CH ₂ str	
	2156	C≡C str	
	1500	p-substituted arom.	
	1248	Si-C str	
Poly(ethynyl styrene)	3280	C≡CH str	5.4c
	3011	Arom. Str	
	2925, 2845	CH ₂ str	
	2055	C≡C str	
	1500	p-substituted arom.	
Poly(ethynyl styrene)	3569	NH str	5.4d
functionalized with	3051	Arom C-H str	
azido-RB	2950, 2871	CH ₃ str	
	2927, 2826	CH ₂ str	
	1653	Amide I	
	1595	C=N str of triazole	
	1524	Amide II	
	1259	N=N str of triazole	
	1176	S=O str	
poly(NHS4VB)	1801	C=O in phase str of imide	5.5b
	1769	C=O out of phase str of imide	
	1738	C=O str of ester	
	1607	p-substituted aromatic	
	1258	Asym. C-O str	
	1205	C-N str, Amide III	
	1026	Sym. C-O str	
	3300	NH str	

Table 5.2. Important vibrational modes and mode assignments for the GATR-FTIR spectra of surface attached initiators and polymers

	3040	Arom C-H str		
	2921, 2853	CH ₂ str		
poly(NHS4VB)	1642	Amide I	5.5c	
functionalized with	1607	p-substituted arom		
Py-N	1532	Amide II		
	1497	Arom ring str		
	1309	Amide III, CN str		

* abbreviations: str= stretch, arom = aromatic

The TMS styrene brush (Figure 5.4b) is fully deprotected using potassium hydroxide/methanol to form the terminal alkyne (Figure 5.4c) by the disappearance of the internal C=C at 2156 cm⁻¹, appearance of the terminal C=C stretch at 2055 cm⁻¹, and C=CH stretch at 3280 cm⁻¹. Functionalization of the brush is demonstrated by the absorbances of RB at 1595, 1259 and 1176 cm⁻¹ representing the C=N, N=N and S=O stretches, respectively.



Figure 5.4. Cascade of TMS polymerization (b) on catechol ATRP initiator surfaces (a), deprotection of alkyne (c) and functionalization with azido RB (d).

Poly(NHS4VB) brushes on TiO₂/catechol surfaces were derivatized as well using a primary amine. The peaks at 1801, 1769, and 1738 cm⁻¹ are carbonyl stretches of the NHS activated ester derivative, while the C-O stretches are visible at 1258 and 1026 cm⁻¹. The peak at 1258 cm⁻¹ is due to the C-N stretch of NHS. Aminolysis is demonstrated by the loss of the activated ester and formation of amide I and II stretching at 1642 and 1532 cm⁻¹, respectively.



Figure 5.5. SI-ATRP of NHS4VB from catechol initiators (a) to form the polymer brush (b) and subsequent derivatization with Py-N (c).

Relative polymer growth using SI-ATRP was further verified using SEM. Figure 5.6 below shows SEM images of PNIPAM, and poly(NHS4VB) at the junction between silicon and titanium oxide surfaces. The image indicates no polymer growth on the SiO₂ with exclusive polymer brush on TiO₂ side. The uneven edges at the seam of the two interfaces are due to the tape mask used to pattern the titanium oxide in the PVD chamber. There is also a "ribboning" effect upon close inspection of the polymer at the SiO₂/TiO₂ junction, which is due to polymer collapse where grafted chains are not forced into an extended conformation due to neighboring chains.⁶¹ The chains therefore extend laterally and develop a random coil configuration at the SiO₂ interface.



Figure 5.6. Scanning electron microscope images of PNIPAM (a & c) and poly (NHS4VB) (b & d) brushes from a top view (a, b) and a side view (c, d) to show exclusive polymerization on the TiO_2 surface.

Elemental analysis using energy-dispersive x-ray spectroscopy (EDX) of the surface indicates a strong presence of carbon and oxygen on TiO_2 in comparison to bare control substrates on both the PNIPAM and poly(NHS4VB) substrates from the polymer brushes.

There is no carbon detected on either SiO_2 substrate, and the oxygen peaks present have the same relative intensity to bare SiO_2 .

Functional polymer brushes were also polymerized from catechol initiators using ring opening metathesis polymerization (ROMP) of norbornene – NHS ester (Nb-NHS). Polymer brush thickness on the titanium oxide surface was 82.7 nm with a contact angle of 67° , while the silicon oxide portion of the substrate had a thickness of 2.4 nm and a contact angle of 58°. Poly(Nb-NHS) thickness was comparable to SI-ROMP of Nb-NHS on octenyl trichlorosilane initiator substrates, used as a control, under identical reaction conditions. Polymer brush segregation between silicon oxide and titanium oxide sections is similar to catechol-ATRP surfaces, where no carbon is detected on SiO₂ by EDX and significant amounts of carbon and oxygen are detected on the titanium oxide side. In addition, ruthenium was detected in the polymer brush matrix. This result is unexpected, since quenching ROMP with ethyl vinyl either should displace the ruthenium catalyst from the chain ends upon addition. The SEM image of poly(Nb-NHs) is in Figure 5.7.


Figure 5.7. SEM image of poly(Nb-NHS) brushes selectively grown on TiO_2 over SiO_2 from top (a) and side (b) perspectives.

Upon SEM characterization of the surface, a heterogeneity in the film was observed, so the polymer brush was rinsed with DMF to remove any physisorbed material that was insoluble in DCM, as has been done previously with poly(NHS4VB) films. Upon rinsing, the film thickness decreased to 42.7 nm with a corresponding contact angle of 55° for the TiO₂ film. GATR-FTIR measurements were taken before rinsing with DMF, and after DMF and DMSO rinsing and are shown in Figure 5.8.



Figure 5.8. GATR-FTIR spectra of Nb-NHS polymer brush on catechol/ TiO_2 surface (a) and after rinsing with DMF (b) and anhydrous DMSO (c) which hydrolyzes the activated ester.

The spectra show significant loss of the NHS ester absorbances at 1801, 1769, and 1738 cm⁻¹ and formation of carboxylic acid bands at 3450, 1670, 1010, and 960 cm⁻¹ representing the OH stretching, C=O and C-O stretching, and OH bending regions, respectively (Figure 5.8b). This rinsing process was repeated using anhydrous DMSO to exclude any possible contamination causing the hydrolysis in the DMF (Figure 5.8c). The DMSO rinsing step removed all NHS ester cites and formed carboxylic acid groups on the brush. We observed similar hydrolytic behavior previously with the formation of

poly(NHS-methacrylate brushes) by ATRP.⁴⁰ A β-alanine linked active ester polymer brush, *N*-methacryloyl-β-alanine *N*-hydroxysuccinimide ester (MAC₂AE), made by free radical polymerization is highly reactive to aminolysis, achieving amide functionalization of the surface within 4 seconds.⁶² In contrast, poly(NHS4VB) takes 7.5 minutes to achieve 70% functionalization.⁴⁰ Differences in reactivity between the activated esters are most likely due to the difference in electrophilicity of the carbonyl to nucleophilic attack. The phenyl group alpha to the NHS ester of NHS4VB provides resonance stability to the carbonyl, decreasing its electrophilicity and making it more stable to hydrolysis. The norbornene NHS esters, like the MAC₂AE and NHS-methacrylate do not have that stabilization and will more readily react in polar conditions. The instability of the NHS ester to rinsing with polar solvents makes aminolysis with poly(Nb-NHS) brushes impractical, as polar solvents are used to insure solvation of the brush and better access to all polymer side chains. For this reason, poly(Nb-NHS) brushes were excluded from further study.

Dual Functional Polymer Brush Scaffold Strategies

The selective SIP of PNIPAM, poly (NHS4VB), and poly (TMS styrene) from a catechol-titanium oxide surface has been demonstrated with several polymerization techniques to fabricate site-specific polymer brushes from the bottom up. The further incorporation of chemical and spatial complexity can be introduced using a second functional polymer brush scaffold. The next section focuses on the development of spatially-resolved poly (NHS4VB) and poly(TMS styrene) brushes that can be sequentially functionalized with amine and azide moieties, respectively.

The central theme to designing a dual orthogonal polymer scaffold is the concept of non-competitive chemistry. The deposition of monolayers, surface initiated polymerization methodologies, and post-polymerization modifications between two patterned oxide regions should function independently of each other to prevent cross-over of reactions, and therefore cross-contamination. The strategies to accomplish noncompetitive growth and functionalization of polymer brushes will be evaluated and discussed. A schematic of the three orthogonal parameters studied are depicted in Figure 5.9.



Figure 5.9. Three controllable parameters for dual polymeric scaffolds: selective dual monolayer formation (a), independent polymerization methods for functional polymer brushes (b) and post-polymerization modification by aminolysis and click chemistry (c).

In the three methods depicted to control orthogonality, the first is the selective deposition of a second initiator monolayer (Figure 5.9a). Initiators containing trichlorosilane, trimethoxysilane, and catechol anchor groups were explored for SiO_2 functionalization and subsequent polymerization. Monolayer deposition control is the foundation of spatially controlled polymer brush growth as the initiators template where polymerization will occur.

The second controllable parameter is the two types of polymerization and monomers used to make the polymeric scaffold (Figure 5.9b). Selection of polymerization mechanisms and monomers must be evaluated critically. There are several parameters, such as initiator, monomers, reaction solvent, temperature, catalysts compounds that need to be compared to ensure that one polymerization system remains inactive and chemically stable to the polymerization conditions of the other. We will discuss and evaluate our two choices of dual polymerization methods: ATRP and nitroxide mediated polymerization (NMP) and successive dual ATRP with styrenic monomers containing active ester and alkyne functionalities.

The final control, as depicted in Figure 5.9c, is post-polymerization modification. Polymers containing both alkynes and activated esters have previously been functionalized orthogonally on the surface and in solution by amines and azides in a one pot reaction.^{4,52,53} While orthogonal polymer brush functionalization is the ideal reaction method, successive functionalization can be performed to functionalize both activated ester and alkyne sites. Attempts at orthogonal and successive functionalizations are described in detail below.

Dual Polymer Brush Surfaces by Formation of Dual Monolayers, Then Successive Surface Initiated Polymerization

Initially, we investigated the combination of ATRP and ROMP for spatially resolved polymeric scaffolds. Both reactions occur rapidly at low temperatures, give thick films within minutes to a few hours. The driving force for polymerization reaction for both polymerization methods are non-competitive: the equilibrium concentration of propagating radicals controlled by the copper-ligand complex of ATRP and the relief of ring strain by the ring-opening of cyclic olefins by a ruthenium catalyst in ROMP. This was demonstrated, however, to be a poor choice for orthogonal surface initiated polymerization methods. An alkyne-functionalized norbornene with a TMS protecting group was initially synthesized for ROMP on the surface to compliment ATRP of However, the TMS-protected alkyne was not bulky enough to prevent NHS4VB. coordination of ruthenium in the Grubbs catalyst (ROMP) to monomer. A bulkier triisopropyl silyl (TIPS) protecting group was also synthesized to make alkyne polynorbornenes, but deprotection conditions to remove the TIPS protecting group required the use of tetrabutyl ammonium fluoride, which also cleaved all polymers from the substrate. A reevaluation of our method was required. A combination of two other controlled polymerization techniques was examined: ATRP and nitroxide-mediated polymerization (NMP). ATRP will undergo controlled radical polymerization by the equilibrium activation and deactivation of the propagating radical from an alkyl halide initiator controlled by a copper-ligand complex. ATRP has a rate acceleration in polar solvents and can be carried out with mild heating or at room temperature. NMP is a stable free radical polymerization, which requires no catalyst, and works well with

styrenic monomers. The initiator consists of an alkoxyamine (Figure 5.9a and 5.9b), which thermally decomposes at the C-O bond to form a reactive radical and 2,2,6,6-tetramethyl-1-piperidinoxyl (TEMPO) radical that is stabilized due to steric hindrance. TEMPO radicals will terminate some of the propagating chains but can be reinitiated, lowering the concentration of propagating radicals, similar to ATRP. Previous studies have demonstrated selective initiation of Y-shaped initiators with an ATRP alkyl halide and a NMP alkoxyamine on each branch and have shown the ability to make mixed polymer brushes by exclusively initiating one initiator, then the other.⁶³⁻⁶⁵

Catechol ATRP initiators on TiO_2 were formed and were then backfilled with chlorosilane NMP initiators to create dual initiator surfaces on TiO_2 and SiO_2 . A summary of the thicknesses and contact angle data of the monolayers is shown in Table 5.3.

Table 5.3. Thickness and contact angle measurements of ATRP and NMP initiators and orthogonal polymerizations of poly(NHS4VB) and poly(TMS styrene), respectively.

SiO₂

	thickness (nm)	contact angle (degrees)	thickness (nm)	contact angle (degrees)
Catechol ATRP init.	0.9	0	4.8	66
Silane NMP init.	4.2	81	6.0	81
Poly(NHS4VB)	15.8	74	67.2	74
Poly(TMS styrene)	128.0	102	75.0	102

TiO₂

The second initiator deposition of the NMP silane make the SiO₂ surface hydrophobic with a contact angle of 81° with a relative monolayer thickness of 4.2 nm. The contact angle of TiO₂ significantly increased from 66° to 80° and increased in thickness by 1.2 nm. This indicated that there is likely some silane NMP initiator on TiO₂. In keeping silane concentration low and limiting deposition time to an hour, the overall SAM increase on TiO₂ was small compared to the monolayer formation on SiO₂. Although there is some cross contamination of initiator, by performing ATRP first, the NMP initiator sites on the TiO₂ surface should be buried, and not propagate the cross contamination to the polymer surfaces. Polymerization of NHS4VB by ATRP yielded 67.2 nm of polymer brush on TiO_2 and 15.8 nm of SiO_2 . This result is unexpected because the reaction conditions of ATRP are at low temperature (50°C) and should not initiate NMP. However, to control for the heat initiating any of the NMP sites, the reaction was repeated at room temperature, giving 37.4 nm of poly(NHS4VB) on TiO₂ and 15.0 nm on SiO_2 . In the mixed polymer brush work conducted by Zhao, they demonstrated no initiation of NMP under ATRP reaction conditions.⁶³ Their control study did not include an alkyl halide initiator, however. The propagating ATRP radical must therefore have a chain transfer effect to the alkoxyamine initiator for NMP and initiate some of the sites, even at room temperature reaction. NMP of TMS styrene was then executed, giving 128 nm of poly(TMS styrene) on TiO₂ and 75 nm on SiO₂ with hydrophobic contact angles of 102° for both substrates. The original poly(NHS4VB) brushes did not fully shield the NMP initiators from polymerizing on TiO₂, evident by the increased thickness change and hydrophobic contact angle increase by 28°. Chemical functionality of the surface was confirmed by GATR-FTIR show in Figure 5.10.



Figure 5.10. Dual initiator ATRP/NMP wafers on SiO_2 (a) TiO_2 (b) and poly(TMS styrene) on SiO_2 (c) poly (NHS4VB) on TiO_2 (d).

The ATRP and NMP monolayer spectra are not significantly different. There is more CH₂ stretching character in the NMP initiators (Figure 5.10a) on SiO₂ and an aryl ether C-O-C stretch present at 1280 cm⁻¹, along with N-O stretching at 1394 cm⁻¹. The ATRP catechol initiator is characterized by C=O stretching at 1645 cm⁻¹ and C-O stretching at 1220 cm⁻¹, as described previously in this chapter. The analysis of the dual polymer surfaces (Figure 5.8c and 5.8d) shows cross contamination between poly(TMS styrene) and poly(NHS4VB) by the C=C and C=O stretches in both spectra (Figure 5.10c and 10d) at 2150 and 1769 and 1738 cm⁻¹, respectively. Polymerization of TMS styrene on TiO₂ may come from two possible sources: the buried NMP initiator, or chain transfer from the alkyl halide chain ends.⁶⁵ Due to the 90 minutes of polymerization time of ATRP, there should not be many alkyl halide sites remaining. Polymer brush thickness plateaus after an hour of reaction due to decreased concentration of propagating radicals on the tethered chain ends, caused by coupling and disproportionation reactions of the proximate radicals.^{40,66} To exclude any chain transfer, however, ATRP polymerization was repeated from two catechol-only TiO₂/SiO₂ substrates and one reaction was quenched with tributyl tin hydride to replace any C-Br chain ends with C-H. The substrates were checked on GATR-FTIR and showed no difference in poly(NHS4VB) spectra in the window of 500 to 3600 cm⁻¹, and no discernable C-Br stretch (550-600 cm⁻¹) was visible in either spectra. NMP silane initiator was then backfilled onto the surface and NMP of TMS styrene was conducted. A summary of film thicknesses is shown in Table 5.4.

Table 5.4. Thickness measurements for ATRP brushes treated with tributyl tin hydride to remove the C-Br initiator chain end and untreated brushes. The brushes were then subjected to NMP initiator deposition and polymerization and showed virtually no difference between the treated substrate and the control.

	Sn-treated brushes		Non-treated polymer brushes			
-	SiO ₂	TiO ₂	SiO ₂	TiO ₂		
-	Thickness (nm)					
Poly(NHS4VB)	1.8	29.4	2.1	30.8		
NMP initiator	7.2	34.8	7.9	34.8		
Poly(TMS styrene)	58.3	79.3	52.6	61.4		

The data in Table 5.4 shows that deactivation of chain ends is not the cause of dual brush cross contamination, and that it is initiation of NMP sites. The change in thickness of the poly(NHS4VB) after poly(TMS styrene) by NMP is similar between the polymer brushes quenched with tin and the control, with the tin-treated TiO_2 surfaces having a larger brush increase after TMS styrene polymerization by 19 nm. The poly(TMS-styrene) on the SiO₂ surfaces of both samples was comparable at 58.3 nm and 52.6 nm.

Dual Polymer Brush Surfaces by Successive Initiator Deposition and Polymerizations using Catechol and Silane Monolayers

While it is ideal to make controlled monolayers for both ATRP and NMP first before polymerization, our initial studies demonstrated that completely segregated polymer brush surfaces are not possible when both initiators are present before polymerization reaction. To remove the possibility of NMP initiation during ATRP, catechol ATRP initiators were first polymerized, then the surface was backfilled with a second initiator to polymerize TMS styrene by NMP. Two strategies for this method will be discussed: time limited deposition of chlorosilane NMP initiator and time limited deposition of methoxy-silane NMP initiator.

Time dependent NMP backfilling with chlorosilane was found to have poor control over selective formation of poly(TMS styrene) on SiO₂. Chlorosilane initiators deposited on the surface for 15, 30, 45, and 60 minute intervals all had cross contamination of poly(TMS styrene) with GATR-FTIR spectra that all resembled Figure 5.10d, with absorbances representing both the activated ester of poly(NHS4VB) and the C=C stretching of the poly(TMS styrene).

Secondary monolayer deposition with a less reactive methoxysilane silane afforded better control for the monolayers. The methoxysilane NMP monolayer was chosen because of decreased reactivity and it is less likely to self-polymerize than chlorosilane.⁶⁷ Also, this compound does not require heat, base catalyst, or long reaction times, such as with ethoxysilanes.⁶⁸ Methoxysilane NMP initiators were deposited on bare SiO₂/poly(NHS4VB) TiO₂ surfaces for 30 minutes to achieve functionalization of the SiO₂ surface but minimize diffusion and functionalization on TiO₂. The initiators, once rinsed were immediately added to the degassed anisole/TMS styrene solution and NMP was performed. Film thicknesses and contact angle measurements for the methoxy NMP substrates are summarized in Table 5.5.

Table 5.5. Polymer brush thicknesses for dual polymer surfaces from catechol/ATRP poly(NHS4VB) on TiO₂ and methoxysilane/NMP/poly(TMS styrene) on SiO₂.

TiO₂

		-	2		
	thickness (nm)	contact angle (degrees)	thickness (nm)	contact angle (degrees)	-
Poly(NHS4VB)	2.3	0	34.5	54	_
Poly(TMS styrene)	15.0	99	40.5	81	
Py-N functionalized	15.8	96	44.2	75	
Azido RB functionalization	14.4	68	44.3	70	

SiO

The poly(TMS styrene) brush thickness was 15 nm after polymerization on SiO₂, while the poly(NHS4VB) brushes film thickness only increased by 6 nm from 34.5 nm to 40.5 nm. While the contact angle of the poly(NHS4VB) did become more hydrophobic after TMS styrene polymerization, from 54° to 80°, no observable C=C stretching was found on the GATR-FTIR of poly(NHS4VB) after NMP polymerization (not shown).

Brush functionalization of poly(NHS4VB) and poly(TMS styrene) was demonstrated in a two-step functionalization. While orthogonal alkyne/azide polymer functionalization has been demonstrated previously, the removal of the alkyne protecting group needed for polymerization proved to be incompatible with NHS ester stability. Deprotection of poly(TMS styrene) polymer brushes require strong base to remove the trimethylsilyl group, which causes base catalyzed hydrolysis of the activated ester. Deprotection methods using TBAF are not feasible due to SiO_2 etching, which causes cleavage of the polymer brush. An alternative deprotection method using silver triflate was attempted, which has been demonstrated to remove alkyne protecting groups chemoselectively under mild conditions.⁶⁹ While removal of the protecting group was confirmed by loss of methyl stretches in GATR- FTIR, the characteristic C=C and C=CH stretches did not appear, indicating that silver was most likely coordinated to the alkyne within the polymer film, preventing azide functionalization of the brush by CuAAC.

Two step functionalization of the poly(NHS4VB)/poly(TMS styrene) was then executed. The substrate underwent aminolysis with aminomethylpyrene (Py-N) to first functionalize the poly(NHS4VB) brushes. There was little change in the SiO₂/poly(TMS styrene) surface, with only an increase of 0.9 nm thickness and a 3° contact angle decrease, while the TiO₂ surface increased in thickness to 44.2 nm, and the contact angle increased from the original poly(NHS4VB) brushes from 54° to 75°. The TMS protecting group on the NMP polymer was then removed by potassium hydroxide in methanol to give poly(ethynyl styrene) with confirmed GATR-FTIR absorbances at 3300 cm⁻¹ and 2051 cm⁻¹ representing the C=CH and C=C stretching regions, similar to the spectra Figure 5.4c. The alkyne pendant group was functionalized with azido-RB under CuAAC conditions. Functionalization yielded a decrease on SiO₂ contact angle of 28° due to the hydrophilic sulfonate groups or aminium groups on the RB, while only a 5° decrease in contact angle for the TiO₂ surface. There was no change in thickness in the TiO₂ surface and a 1.4 nm decrease in film thickness for the poly(ethynyl styrene)

functionalized with azido RB. Due to the very small film thickness of poly(TMS styrene) from methoxysilane NMP initiators (15nm) as compared to the polymer from chlorosilane NMP initiators (128 nm), it is possible that grafting density of the poly(TMS styrene) is much lower than with the chlorosilane NMP initiator surface. Without densely packed neighboring chains to force chain extension of the grafted brush, the polymer is more likely to exist in the aforementioned "mushroom" regime where chains exist in their random coil configuration on the surface. Therefore, the chains can rearrange themselves once functionalized, and the large change in thickness relative to molecular weight increase of the pendant chain will not be observed. GATR-FTIR spectra for Py-N and azido-RB functionalized SiO₂ and TiO₂ surface are shown in Figure 5.11.





For the catechol/methoxy substrates, the attachment of the Py-N is evident by the amide stretches at 1643 and 1525 cm⁻¹ for TiO₂ surfaces. The azido-RB functionalization is evident on the silicon oxide surface by the C=N stretching at 1594 cm⁻¹ and the S=O stretching at 1175 cm⁻¹. There is some small absorbance on TiO₂ in the Py-N bands which may indicate cross contamination and functionalization of azido-RB on both sides. This may be due to physisorbed dye on the brush, as the overall segregation of the two fluorescent dyes is readily visible by fluorescence microscopy shown in Figure 5.12.



Figure 5.12. Selective functionalization of catechol initiated Poly(NHS4VB) surface with Py-n (a) and a methoxysilane initiated poly(ethynyl styrene) with azido-RB (b). These two images were taken on a fluorescent microscope with a DAPI filter (445-450 nm emission)(a) and a GFP filter (525-550 nm emission)(b).

The Py-N dye appears on the TiO_2 surface exclusively and shows the segregation between the two polymerized oxide surfaces. The Rhodamine B dye also appears to selectively bind the SiO₂ surface, although more dye does appear sporadically on the TiO₂ surface. The coverage of RB on TiO₂ suggests physisorption over chemisorption. Polymer brush growth of poly(TMS styrene) on TiO_2 would be indicated by a much more even coverage of azido-RB functionalization as the methoxy NMP initiator diffuses into the brush matrix.

Dual Polymer Brush Surfaces by Successive Initiator Deposition and Polymerizations using Tandem Deposition of Catechol Monolayers

Dual polymerization methods can also include tandem polymerizations by the same mechanism, providing no active initiator remains at the polymer brush chain ends prior to the initiation of the second initiator monolayer. Tandem polymerizations are inherently non-competitive, due to identical reaction conditions, only differing by the addition of a different monomer. We have demonstrated no further chain transfer or initiation occurs from poly(NHS4VB) polymer brushes after 90 minutes of reaction time from SIP from the ATRP catechol initiator. We decided to evaluate the efficacy of using catechols selectively to deposit successive ATRP initiators to form orthogonal functional surfaces. It has been previous demonstrated that PEG chains with catechol end groups selectively bind the most PEG to a surface when solution pH which is the same as the oxide surface's IP.³⁶ Prior results from the literature indicate no TiO₂ catechol adsorption occurred at pH 2, the IP for SiO₂. While studies demonstrated a low affinity of catechol for silica and glass, selective functionalization of dopamine can occur on SiO_2 over TiO₂ at acidic pH. We therefore explored using pH-selective deposition of catechol initiators to backfill SiO₂ surfaces with a second catechol ATRP initiator after polymerization. Poly(NHS4VB), while more stable than other activated NHS esters, will not tolerate pH 2 conditions and will hydrolyze in acidic conditions. Therefore, poly(NHS4VB) was functionalized with Py-N prior to catechol deposition. Film

thickness measurements and contact angles for dual catechol substrates are consolidated in Table 5.6.

Table 5.6. Thickness and contact angle measurements for SiO_2 and TiO_2 orthogonal polymer surfaces by sequential deposition of ATRP catechol monolayers.

	SiO ₂		TiO ₂		
	thickness (nm)	contact angle (degrees)	thickness (nm)	contact angle (degrees)	
Poly(NHS4VB)	1.6	58	36.6	55	
Py-N functionalized	1.8	38	52.6	80	
pH 2 ATRP Catechol	2.5	47	58.3	89	
Poly(TMS styrene)	15.7	83	61.6	88	
Azido RB functionalization	14	57	60.9	75	

Py-N functionalized substrates were soaked 24h in catechol/water solution adjusted to pH 2 by HCl. The SiO₂ substrates show an increase in film thickness of 0.7 nm, while the TiO₂ surface increases by 5.7 nm. SiO₂ and TiO₂ contact angles increased by 8°. This data indicates possible catechol formation on both oxides, except the thickness increases post-poly(TMS styrene) infer the opposite result. The poly(TMS styrene) brushes on SiO₂ are 15.7 nm thick while the TiO₂ surface Py-N functionalized brushes only increase by 3 nm. Contact angles of SiO₂ and TiO₂ reflect the hydrophobic TMS styrene and Py-N functionalities, at 83° and 88° degrees on the SiO₂ and TiO₂ sides respectively. Increasing the hydrophobic character of the TiO₂ sides by polymerization of TMS styrene, however, would likely increase the hydrophobicity of the surface to a higher contact angle, while the contact angle of the Py-N functionalized surface does not change with TMS styrene ATRP. Finally, deprotection of the alkyne brush and CuAAC with azido RB yield a decrease in contact angle of for SiO₂ and TiO₂ of 26° and 13°, respectively. The decrease in contact angle on TiO₂ is due to solvation effects in water as a slight decrease in film thickness of 0.7 nm is observed. The SiO₂ thickness decreases by 1.7 nm, due to poor surface coverage of the second catechol initiator.

Dual catechol brush functionalization is also characterized by FTIR in Figure 5.13. Azido-RB functionalization of poly(ethynyl styrene) on silicon oxide is highlighted by the C=N stretch observed at 1594 cm⁻¹, the N=N stretch at 1258 cm⁻¹, and the S=O stretch at 1180 cm⁻¹ on the SiO₂ wafer (figure 5.11a). The Py-N absorbances on the TiO₂ half of the substrate are weak and broad in the spectra, at 1642 and 1532 cm⁻¹, with a shoulder absorbance at 1600 cm⁻¹. The shoulder absorbance resembles the C=N stretch of azido-RB, making the IR conformation of exclusive RB functionalization on TiO₂ inconclusive. This result is clarified, however, in the fluorescence microscopy images of the dual functionalized surfaces in Figure 5.14. Despite from a minimal amount of sporadic, physisorbed material, Py-N and azido-RB remained segregated on the silicon and titanium oxide surfaces.



Figure 5.13. GATR-FTIR spectra of Py-N and azido-RB attachment to TiO_2 (b) and SiO_2 (a) surfaces, respectively.



Figure 5.14. Selective functionalization of catechol initiated poly(NHS4VB) surface with Py-N (a) and catechol initiated poly(ethynyl styrene) with azido-RB (b). These two images were taken on a fluorescent microscope with a DAPI filter (445-450 nm emission)(a) and a GFP filter (525-550 nm emission) (b).

The lower wavelength excitation of Py-N at 317 nm shows the functionalized poly(NHS4VB) brushes (Figure 5.14a) exclusively on the TiO₂ surface. The Rhodamine B dye also appears to selectively bind the SiO₂ surface, although the contrast between the SiO₂ and TiO₂ substrate surfaces is not as visible as with the Py-N dye. This contrast is mostly likely due to the low surface coverage of poly(ethynyl styrene). The small, disperse amount of RB dye emission observed on the TiO₂ suggests no chemisorption of the dye. There is negligible contrast between the Py-N film and the bare surface as observed by the scratch in the TiO₂ polymer substrate (Figure 5.14b) further demonstrating RB functionalization exclusively on the SiO₂/poly(ethynyl styrene) brush.

Conclusions

In summary, we have demonstrated the ability to selectively grow controlled, specialized polymers from the orthogonal deposition of a catechol initiator on titanium (IV) oxide over silicon oxide. Secondary orthogonal functionalization of the remaining SiO₂ surface has proven challenging, but can be achieved by deposition of either a methoxysilane NMP initiator or second catechol ATRP initiator monolayer onto the surface. Polymer brush substrates containing spatially resolved patterns of poly(NHS4VB) and poly(TMS styrene) were fabricated. Post-polymerization modification of the polymer brush surface shows excellent fidelity between the dual polymer brush functionalizations with little cross functionalization, although the aminolysis and cycloaddition reactions must be carried out in two successive reactions, and not on one orthogonal reaction step.

Surface coverage of the second initiator deposition backfilled initiator needs to improve to achieve larger film thicknesses for secondary polymerization of TMS styrene.

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Future studies need to include further investigations of surface initiated polymerization from other oxide surfaces, including aluminum oxide and niobium oxide, which have a stronger affinity for catechols than SiO_2 , but at least a two order of magnitude difference in isoelectric point than TiO_2 to tune catechol adsorption.

In the future, further optimization of orthogonal surface functionalization using derivatizable polymer brushes will allow nanostructured surfaces and 3D architectures to have site-specific patterning of chemical functionality from the bottom up, making customizable nanotechnology for applications in materials science and medicine.

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CHAPTER 6

CONCLUSIONS AND OUTLOOK

Conclusions

This dissertation has introduced and described the synthesis of a controlled, functionalizable polymer brush scaffold based on poly(n-hydroxysuccinimide 4-vinyl benzoate) (poly(NHS4VB)). Chapter 1 is a literature review on polymer thin film interfaces, focusing on the unique properties and advantages of polymer brushes grafted from the surface. Various controlled polymerization mechanisms were introduced to describe different methods to make polymers with controlled rates of reaction and low polydispersity. Post-polymerization modification strategies were discussed, focusing on chemical and biological conjugation of the polymer repeat unit, as well as polymer brush patterning.

Chapter 2 detailed the design and synthesis of poly(NHS4VB) brushes using surface-initiated atom transfer radical polymerization with block copolymerization capability. Using a living radical polymerization method, film thickness can be controlled with polymerization time and the polymer chains can be reinitiated easily to form various block copolymer microenvironments. The active ester brushes rapidly undergo quantitative aminolysis with small molecule primary amines on the order of minutes. Post-polymerization modification of poly(NHS4VB) brushes can generate surfaces with high functionalization densities of 25 nmol/cm².

Chapter 3 demonstrated the surface immobilization of cyclopropenone-protected dibenzocyclooctynes on the active ester polymeric scaffold for top down control of surface functionality by photoactivation. Upon UV irradiation, the cyclopropenone decarbonylates, leaving the high energy cyclic alkyne for catalyst free cycloaddition with azides. The decarbonylation reaction occurs quickly and quantitatively with low power UV irradiation. This photoactivated surface platform allows the creation of multifunctional surfaces with spatially resolved chemical functionality, as demonstrated by photoactivation of polymer brush regions as small as 10 microns.

Chapter 4 investigated polymer brush post-polymerization rates for derivatized active ester brushes with different reactive alkynes for Sharpless-type alkyne/azide "click" chemistry using either a high energy cyclooctyne or copper-catalyzed alkyne/azide cycloaddition (CuAAC). The time-dependent functionalization offers a straightforward route to a densely packed functional polymer surface with azide reactivity under mild conditions. Click kinetics for catalyst free cycloaddition and conventional CuAAC demonstrate pseudo-first order limited rates that are 40 to 60 % slower within a polymer brush than corresponding reactions in solution. The relative rates of reaction of the reactive alkynes show a similar ratio between solution reaction and post-polymerization modification rates, demonstrating the negligible effect of a densely packed polymer brush scaffold on the mechanism and geometry of the reaction. Diffusion into the brush matrix does not impact the rate of reaction as long as the azide concentration is sufficiently high. Initial studies on brush functionalization using low azide concentration indicate a diffusion limited rate regime for the last 10% of the alkyne

sites on the brush. The diffusion limited rate factor is constant relative to pseudo-first order reaction kinetics and slows the reaction rate by 75%.

Chapter 5 applies controlled active ester and alkyne polymer brushes to the development of spatially-controlled polymer interfaces from the bottom up. The selective, orthogonal deposition of a catechol–based initiator on titanium (IV) oxide over silicon oxide permits active ester polymerization only in areas templated with TiO₂. Secondary surface functionalization of the remaining silicon oxide sites was demonstrated with protected poly(ethynyl styrene) to functionalize brushes by CuAAC. Post-polymerization modification of the polymer brush surfaces demonstrates spatial control, leading to excellent fidelity between the dual polymer brush functionalizations with little cross-contamination.

Future Work

This dissertation has addressed fundamental studies to fabricate well-controlled polymer scaffolds for post-polymerization modification with small molecule amine and azide functionality. Current work in our laboratory is underway to incorporate poly(NHS4VB) brushes on gold substrates for *in situ* determination of binding affinities with different chemical and biological analytes. The substrates will be scanned with surface plasmon resonance imaging (SPRi) to measure the relative change in reflectivity on the SPR curve, representing a binding event on the surface.

Further studies on diffusion limited functionalization kinetics with poly(NHS4VB) brushes and derivatives needs to be performed. As illustrated in Chapter 4, low concentration of analyte (500 μ M) in solution effect the final 10% of brush functionalization. A series of brush thicknesses, analyte concentrations, and types of

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solution analytes need to be studied to completely evaluate the diffusion limited regime for brush functionalization. The diffusion-limited first order rate equation can then be derived with an adequate number of samples, and better temporal control can be optimized for post-polymerization functionalization of polymer brush scaffolds. In optimizing better temporal control, further 3D polymer brush architectures can be explored, such as the formation of a polymer brush functionalization gradient, or an immobilized polymer bottle brush.

With regards to the dual functional polymer brush studies in chapter 5, greater surface coverage of the second initiator deposited on the silicon oxide patterned surfaces will achieve larger film thicknesses for secondary polymerizations. Future studies need to include investigations of surface initiated polymerization from other oxide surface combinations, including aluminum oxide and niobium oxide, which have a stronger affinity for catechols than SiO₂. They also have at least a two order of magnitude difference in isoelectric point than TiO₂ to tune catechol adsorption. Synthesis of a nitroxide-mediated polymerization initiator with a catechol end group will also aid in making thicker secondary functional polymer brush films in a more controlled manner than the silane initiators studied. The incorporation of dual functional polymer brushes can then be fabricated from micro-and nano-scale 3D structures of patterned oxides to demonstrate site-selective functionalization on a small scale. Only when the ability to generate and control polymer brushes on the nanoscale is demonstrated can we fully evaluate the impact that nanostructured polymer interfaces have on larger, interdisciplinary fields.

Final Remarks

As the applications of medical, electronic, and materials technology become more interdisciplinary, polymer brushes provide a durable and customizable interface between surfaces and the macroscopic environments they aim to influence. This dissertation is a fundamental study in developing a universal scaffold to create customizable and robust polymeric interfaces. An easily modified polymeric interface not only has applicability for commercialization, but can lead to increased interdisciplinary collaborations in academia. The ability to easily customize a polymeric thin film can lead to new polymer conjugate hybrid materials, where the interfacial control can improve the performance of bulk materials in new chemical and biological environments.