

ABSTRACT

POTTLE, DAVID WILLIAM. Synthesis of Poly(Maleic Anhydride-co-ETEG) Polymer Brushes and Thin Films. (Under the direction of Dr. Christopher B. Gorman).

Shortly after biomedical devices are implanted into people, proteins begin to adsorb to the surface. This process is called biofouling and over time, the fouling can lead to device deactivation and rejection by the body. In order to combat this, it is proposed that coatings with biodegradable polymers could be engineered to prevent protein adsorption. The group has previously worked on synthesizing polyester brushes and studied their degradation and their resistance to protein adsorption. It was found that these polyester brushes degraded within a week and had some antifouling capabilities. A previous group member established that it was possible to grow a polyester brush from silicon wafers with a grafting through ring opening copolymerization utilizing a $((\text{Et})\text{AlCl}_2)$ catalyst with succinic anhydride and a pegylated epoxide called ETEG. This reaction allowed for moderate brush growth but complete removal of the brush after 2 hours.

A $\text{Cr(III)Cl}(\text{salen})$ catalyst has been shown by Coates to copolymerize various anhydrides with various epoxides at moderate temperatures, very little ether linkages and high molecular weights. This catalyst was chosen because of its ability to copolymerize maleic anhydride and a pegylated epoxide. This reaction was carried out and gave polymers of around 4 KDa which was much lower than expected. Nevertheless, this molecular weight was enough to proceed with grafting through polymerizations. To accomplish this, surface bound epoxy was attached to silicon wafers and then the polymerization was carried out. All experiments at different molarities using the $\text{Cr(III)Cl}(\text{salen})$ catalyst produced polymer in

solution of reasonable molecular weight, while failing to product any polymer brush.

Multiple attempts were made to replicate the thickness of the surface bound epoxy groups as reported by the previous group member. All of these attempts failed to match the reported thickness of 5 nm. Finally, the grafting through ring opening copolymerization of maleic anhydride and ETEG was attempted using the $((\text{Et})\text{AlCl}_2)$ catalyst in order to directly replicate previous experiments. This resulted in a moderate brush growth of 1-3 nm which was much less than the previously reported 3-14 nm. The results indicate that the Cr(III)Cl(salen) catalyst is unable to perform grafting through on the surface.

These failures led to exploring other ways to attach a polyester to the surface. Another group in the Chemical Engineering department came up with an effective way to attach a surface bound benzophenone (BP-silane) moiety to the surface. Premade blends of poly(Ma-co-ETEG) and BP was spun cast onto the BP-silane wafers and crosslinked. This resulted in poor quality wafers due to phase separation but nevertheless, successfully anchored the polymer network to the surface even after being washed multiple times. It was determined that the BP needed to be incorporated into the polymer chain and this was accomplished by synthesizing an epoxy attached with BP (EBP). Multiple polymers were synthesized by adding different percentages of EBP into the polymer (1%, 2.5%, 10%, 25%) and spin coating these onto BP-Silane wafers followed by crosslinking. These wafers were then subjected to degradation studies at different pHs (6.4, 7.4, 8.2) and to a solution of bovine serum albumin to study protein adsorption. The degradation of the 1% EBP feed ratio was rapid, the 2.5% EBP feed ratio was modest and the 10% and 25% EBP feed ratios

hardly degraded over the time scales studied. However, all of the polymer films showed significant protein adsorption within a few days of stirring in solution.

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Synthesis of Poly(maleic anhydride-co-ETEG) Polymer Brushes and Thin Films

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Chemistry

Raleigh, North Carolina

2018

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Chapter 1 Introduction

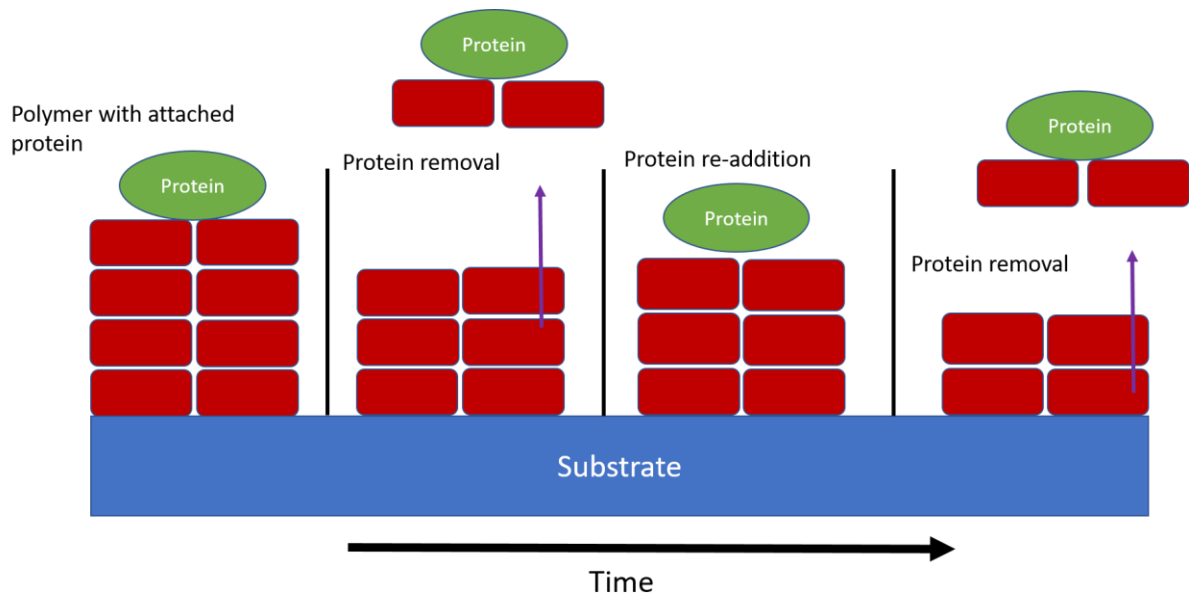
1.1 Biofouling and Protein Adsorption Background

1.1.1 Biofouling and Protein Adsorption with respect to Implantable Medical Devices

The healthcare industry has advanced very rapidly over the past century. One of the outcomes of this advancement is implantable medical devices.¹⁻³ Some examples of implantable medical devices are pacemakers, bone implants, and insulin pumps. For example, insulin pumps work by sensing a target molecule and converting that to an electrical signal, which is then converted into meaningful data by a recorder. One of the major issues of these devices, regardless of type, is the fact that foreign bodies are susceptible to protein adsorption and biofouling.⁴⁻⁶

Biofouling of a sensor occurs when it accumulates enough proteins on the surface that it is deactivated and thus unable to detect the target molecule. This process begins immediately after the device is implanted, first with protein adsorption and then accumulation of cells. Once the device is sufficiently fouled the device can fail, causing it to be removed or replaced. Removal is an extreme inconvenience to the patient and can be life threatening in some cases.^{7,8} One way to prevent fouling is to engineer the devices so that they contain self-cleaning entities.^{9,10} Also, devices can be coated with antifouling¹¹ or antimicrobial materials.^{12,13} One downside of the coatings is that the amount required to be effective might prevent the device from being able to detect its target molecule. One possibly way to circumvent this problem might be to employ a degradable coating so that over time, the coating falls off of the device shedding any adsorbed protein and possibly allowing it to be re-activated. An example of this idea is shown below in Scheme 1.1.

Scheme 1.1. Illustration of a surface coated with a degradable polymer.



1.1.2 Protein Adsorption

One of the major causes of protein adsorption is hydrophobic interactions and the charged states of proteins. Protein adsorption is the first step in the biofouling process and thus is the root cause of device deactivation.¹⁴⁻¹⁶ This process works by the attraction of the hydrophobic interiors of proteins to hydrophobic materials in a hydrophilic environment. This process is enthalpically favored. Overtime, this attraction will lead to the accumulation of proteins on the surface of the device. Additionally, if a surface has the opposite charge of a protein, then electrostatic interactions will also lead to protein adsorption. There are two types of adhesion to the surface, physisorption which is interaction between protein and substrate via weak, non-covalent, reversible interactions and chemisorption, which is interaction between protein and substrate via strong, covalent, irreversible interactions.¹⁷

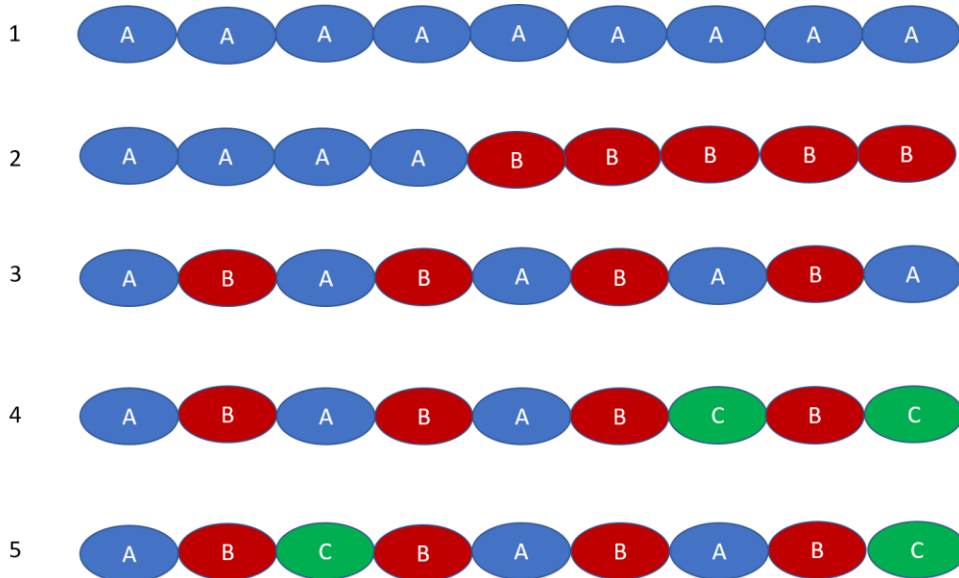
1.2 Polymeric Surfaces Background

1.2.1 Introduction to Polymers

Polymers are a class of materials that consist of a repeating unit. The repeat unit is called the monomer. Polymers are a very important class of materials and make up everyday items such as tires, Teflon, nylon and plastics.

There are two main types of polymers: homopolymers and copolymers. Homopolymers consist of one repeating unit throughout the entire polymer while copolymers consist of two or more repeating units. Copolymers can have very different arrangements. An illustration of different types of copolymers is shown below in Scheme 1.2.

Scheme 1.2. Different Types of Polymers



Example 1 illustrates a homopolymer. Example 2 shows a block copolymer while example 3 is a perfectly alternating copolymer. Example 4 shows a gradient copolymer

where there is a preference to react with monomer A over monomer C. This type of polymer is formed when all of monomer A is depleted and, then monomer C is used to further grow the chain. Example 5 shows a random copolymer where there is no preference for A over C.

There are two major pathways for a polymerization to occur: step-growth and chain-growth. Step-growth occurs when monomer A reacts with monomer B to form AB. The AB dimer can then react with another AB to form ABAB. This process continues until the polymer has reacted with all of the available monomers. The weights of these polymers do not increase appreciably until the very end of the reaction. Another conceivable way to get ABAB alternating polymer is a chain-growth process, where monomer A reacts with monomer B forming AB with a reactive chain end that can add A, then B, etc. This process results in a polymer where the molecular weight of the chains increases linearly as the monomer is depleted.

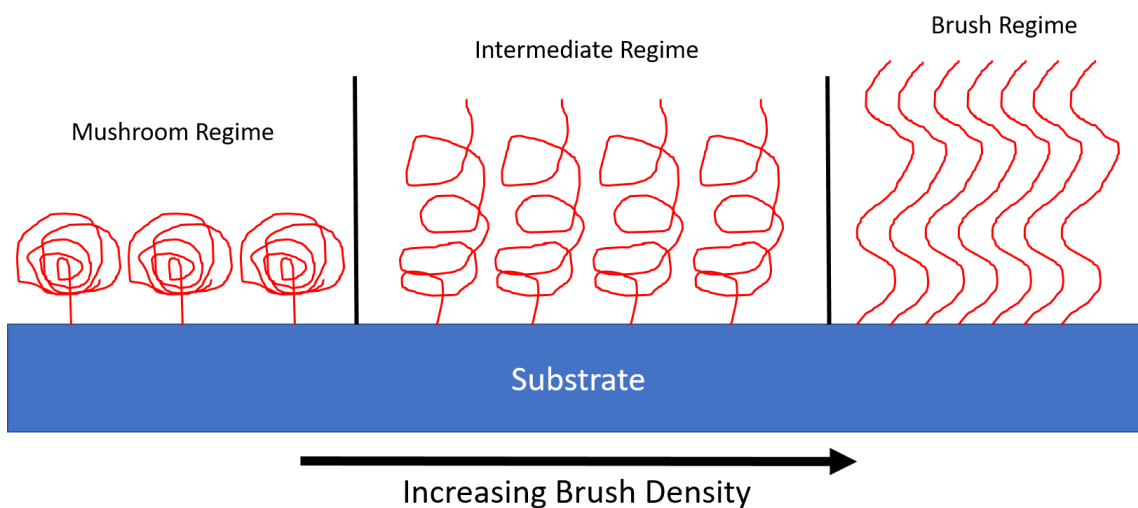
The mechanism of chain-growth polymerizations consists of three steps: initiation, propagation, and termination. Initiation is the activation of a monomer, usually by a catalyst. Propagation is the sequential addition of monomers to the growing polymer chain end. Termination is the deactivation of the active polymer chain end. Deactivation, while rare, can occur from many sources and can happen when two reactive sites of two polymer chains react, thus killing the active sites. This process is called recombination. More commonly, reactive contaminants such as water, oxygen, and other impurities can deactivate the propagating site.

The propagating site in chain-growth polymerizations is frequently an ion, a radical or an organometallic complex. Ionic polymerizations have a narrow range of monomer choice because the propagating chain must be stabilized by electron donating groups in cationic polymerizations or by electron donation withdrawing groups in the case of anionic polymerizations. Radical polymerizations have much more monomer choices than ionic polymerizations and work by generating radicals on each monomer which then propagates.¹⁸

1.2.2 Polymer Brushes

Polymer attachment to a surface is called grafting and involves the covalent attachment of the polymer chain to the surface.¹⁹ Polymer chains grafted to a surface are referred to as polymer brushes. Brushes can have different morphologies depending on how they are formed. An example of the different morphologies can be seen below in Scheme 1.3.

Scheme 1.3. Different Morphologies of Polymer Brushes



When the polymer is synthesized in solution and then attached to the surface, typically this leads to the formation of a mushroom regime. This is called “grafting to” and typically, the individual polymer chains are not able to pack very efficiently resulting in a low density of polymer. Conversely, if a reactive initiator is present on the surface, the polymer can grow up from the surface by adding monomer that is in solution. This process tends to form chains in the more densely packed, brush regime and is called “grafting from”. Both of these processes create polymer brushes where the end of each polymer chain is anchored covalently to the surface. In contrast, a newer approach called “grafting through” involves a combination of “grafting to” and “grafting from”. This process begins with the growth of polymer in solution. Occasionally, the propagating chain encounters a monomer unit that is covalently tethered to the surface. Incorporation of that unit followed by additional propagation by adding more monomers (mostly from solution) grafting the polymer to the surface at some point or points in the middle of the chain.²⁰⁻²³ This process will be discussed more fully in Chapter 2.

Polymer brushes allow for the creation of surfaces with specifically engineered properties. For example, polymer brushes have been made that provide antifouling, adhesive, and antimicrobial properties to a surface. Previous group members have been able to create polymer brushes made of poly(caprolactone) (PCL), poly(glycolic acid) (PGA), and poly(lactic acid) (PLA). These brushes were attached to the surface by utilizing the surface hydroxy groups on silicon wafers.²⁴⁻²⁶ Additionally, poly(succinic anhydride-co-ETEG) was grafted through a surface using surface bound epoxy groups and an ((Et)AlCl₂) catalyst.²⁷

1.2.3 Spin Coating

One way to physisorb polymers to a surface is called spin coating. Spin coating involves synthesizing polymers in solution and then depositing a solution of polymers onto a surface. The surface is then spun rapidly to remove excess polymer and then the solvent is allowed to evaporate. A more detailed description of spin coating is given in Chapter 3.

1.3 Achieving Protein Resistance

Achieving protein resistance has proved to be an elusive problem. The electrostatic interactions and hydrophobic interactions between the substrate and the protein must be disrupted. At first, it was thought that coating surfaces with hydrophobic moieties would work. However, this proved to actually worsen the problem. From there, non-ionic hydrophilic coatings were attempted and this approach has been shown to work much better. Hydrophilic coatings work by attracting water molecules toward the surface, creating a water interfacial layer and make the surface feel similar to bulk water. This property is what is attributed to achieving protein resistance.^{28,29}

In order to achieve the interfacial water layer, a multitude of polymers have been explored. Two of the most commonly studied types are zwitterions and oligoethylene glycol (OEG) moieties. Zwitterions are molecules that have discrete charges but overall are neutral. For example, one end of the molecule might be negative while another group has a positive charge. Zwitterion coatings run into the problem of undesirable electrostatic interactions between the charged nature of the zwitterions and the proteins.³⁰ OEG moieties have been shown to resist proteins very effectively. To start, they are hydrophilic

and thus create a very stable interfacial water layer. Additionally, they are uncharged unlike zwitterions and avoid electrostatic interactions. Both of these qualities allow for desirable protein resistance.¹¹

1.4 Project Overview

This project involves synthesizing a protein resistant, degradable polymer that can be attached to a surface. In order to achieve protein resistance, the degradation is envisioned to be a mechanism for the removal of protein that inevitably adheres to the surface over time. Triethylene glycol (TEG) was incorporated into the polymer by attaching this to one of the monomers. To synthesize the polymer, a ring opening co polymerization (ROCOP) mechanism with a Cr(III) salen catalyst was utilized to give a perfectly alternating copolymer. The co-monomers for this were maleic anhydride and TEG modified epoxide. For polymer brush synthesis, an epoxide was bound to the surface in an attempt to allow for a grafting through mechanism. For spin coating thin films, a surface photolinker was deposited on the surface and a photocrosslinking agent was incorporated into the polymer chain. This allowed for a polymer to be spun coated on the surface and then photocrosslinked to itself and to the surface. Finally, the degradation of these thin films was studied both in the presence and absence of protein to determine of protein adsorbed to them.

1.5 References

- (1) Joung, Y.H.; Development of implantable medical devices: from an engineering perspective. *Int. Neurorol. J.* **2013**, *17* (3), 98–106.
- (2) Dakurah, M.N.; Koo, C.; Choi, W.; Joung, Y.H.; Implantable Bladder Sensors: A Methodological Review. *Int. Neurorol. J.* **2015**, *19* (3), 133– 141.
- (3) Klonoff, D.C.J.; The Benefits of Implanted Glucose Sensors. *Diabetes Sci. Technol.* **2007**. *1* (6), 797–800.
- (4) Tada, S.; Inaba, C.; Mizukami, K.; Fujishita, S.; Geminei-Ide, M.; Kitano, H.; Mochizuki, A.; Tanaka, M.; Matsunaga, T.; Anti-Biofouling Properties of Polymers with a Carboxybetaine Moiety. *Macromol. Biosci.* **2009**. *9* (1), 63–70.
- (5) Harding, J.L.; Reynolds, M.M.; Combating Medical Device Fouling. *Trends Biotechnol.* **2014**, *32* (3), 140–146.
- (6) Follstaedt, S. C.; Last, J. A.; Cheung, D. K.; Gourley, P. L.; Sasaki, D. Y. Protein Adhesion on SAM Coated Semiconductor Wafers: Hydrophobic Versus Hydrophilic Surfaces: Technical Report; SAND2000-3016; Sandia National Laboratories: Albuquerque, New Mexico, 2000.
- (7) Petrone, L.; Molecular Surface Chemistry in Marine Bioadhesion. *Advances in Colloid and Interface Science* **2013**, 195-196.
- (8) Harding, J.L.; Reynolds, M.M. Combating Medical Device Fouling. *Trends in Biotechnology* **2014**, *32*, 140–146.

- (9) Urban, M.W.; Handbook of Stimuli-Responsive Materials; Wiley-VCH Verlag: Weinheim, Germany, 2011.
- (10) Urban, A. M.; Urban, M. W. *Stimuli-Responsive Macromolecules and Polymeric Coatings*. In *Stimuli-Responsive Polymeric Films and Coatings*, ACS Symposium Series; Urban, M. W., Ed.; American Chemical Society: Washington, D.C., 2005; pp 1–25.
- (11) Hucknall, A.; Rangarajan, S.; Chilkoti, A.; In Pursuit of Zero: Polymer Brushes that Resist the Adsorption of Proteins. *Adv. Mater.* **2009**. *21* (23), 2441–2446.
- (12) Munoz-Bonilla, A.; Cerrada, M. L.; Fernandez-Garcia, M.; *Polymeric Materials with Antimicrobial Activity: From Synthesis to Applications*; The Royal Society of Chemistry: Cambridge, UK, 2014.
- (13) Lagaron, J.M.; Ocio, M.J.; Lopez-Rubio, A.; *Antimicrobial Polymers*; John Wiley & Sons, Inc.: Hoboken, New Jersey, 2012.
- (14) Haskard, C.A.; Li-Chan, E.C.Y.; Hydrophobicity of Bovine Serum Albumin and Ovalbumin Determined Using Uncharger (PRODAN) and Anionic (ANS⁻) Fluorescent Probes. *J. Agric. Food Chem.* **1998**. *46* (97), 2671–2677.
- (15) Boujday, S.; Bantegnie, A.; Briand, E.; Marnet, P.G.; Salmain, M.; Pradier, C.M.; In-Depth Investigation of Protein Adsorption on Gold Surfaces: Correlation the Structure and Density to the Efficiency of the Sensing Layer. *J. Phys. Chem. B.* **2008**. *112* (21), 6708–6715.
- (16) Kim, J.J.; Protein Adsorption on Polymer Particles. *Biomed. Mater. Res.* **2002**. *21* (2), 4373–4381.

- (17) Pasche, S. Mechanisms of Protein Resistance of Adsorbed PEG-Graft Copolymers. Ph.D. Dissertation, Swiss Federal Institute of Technology Zurich, 2001.
- (18) Odian, G. Principles of Polymerization, 4th Ed.; John Wiley & Sons, Inc.: Hoboken, New Jersey, 2004.
- (19) Krishnamoorthy, M.; Hakobyan, S.; Ramstedt, M.; Gautrot, J.E.; Surface-Initiated Polymer Brushes in the Biomedical Field: Applications in Membrane Science, Biosensing, Cell Culture, Regenerative Medicine and Antibacterial Coatings. *Chem. Rev.* **2014**. 114, 10976–11026.
- (20) Li, B.; Yu, B.; Ye, Q.; Zhou, F. Tapping the Potential of Polymer Brushes through Synthesis. *Acc. Chem. Res.* **2015**, 48 (2), 229–237.
- (21) Stamm, M. Polymer Surfaces and Interfaces, 1st. Ed.; Springer-VBH: Berlin, Germany, 2008.
- (22) Henze, M.; Mädge, D.; Prucker, O.; Rühle, J.; “Grafting Through”: Mechanistic Aspects of Radical Polymerization Reactions with Surface-Attached Monomers. *Macromolecules.* **2014**. 47 (9), 2929–2937.
- (23) Brittain, W. J.; Minko, S.; A Structural Definition of Polymer Brushes. *J. Polym. Sci. Part A Polym. Chem.* **2007**. 45 (16), 3505– 3512.
- (24) Crawford, K. E. Growth and Degradation of Poly(glycolic acid) and Poly(ϵ -caprolactone) Brushes. M.S. Thesis, North Carolina State Univ., 2011.
- (25) Hu, X. Investigation of Growth and Degradation Behaviors of Biodegradable Polyester Brushes and Their Applications. Ph.D. Dissertation, North Carolina State Univ., 2013.

- (26) Hu, X; Gorman, C.B.; Resisting Protein Adsorption on Biodegradable Polyester Brushes. *Acta. Biomaterialia*. **2014**. 3497-3504.
- (27) Daye, A.J.; Synthesis of Protein-Resistant, Biodegradable Polymer Brushes, M.S. Dissertation, North Carolina State Univ., **2016**.
- (28) Kochkodan, V.; Johnson, D. J.; Hilal, N.; Polymeric Membranes: Surface Modification for Minimizing (Bio)Colloidal Fouling. *Advances in Colloid and Interface Science* **2014**, 206, 116–140.
- (29) Song, W.; Mano, J. F.; Interactions Between Cells or Proteins and Surfaces Exhibiting Extreme Wettabilities. *Soft Matter* **2013**, 9, 2985.
- (30) Shao, Q.; Jiang, S. Molecular Understanding and Design of Zwitterionic Materials. *Adv. Mater.* **2014**, 27, 15–26.

Chapter 2 Poly(Maleic anhydride-co-epoxymethoxytriethylene glycol) Brushes

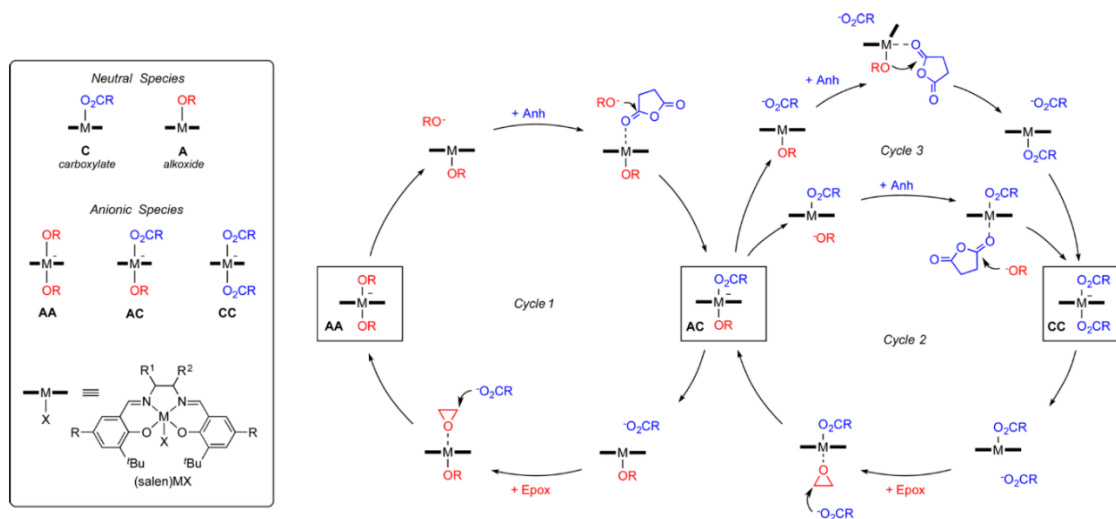
2.1 Introduction

2.1.1 Ring Opening Co-polymerization

Polyesters are a large class of commercial polymers used in everything from plastic bottles to biomedical devices.¹ They are an appealing alternative class of polymers compared to petroleum based polymers because of their renewable source, high biocompatibility and ability to degrade into non-toxic moieties.²⁻⁵ Currently, the most common method for making polyesters is a step-growth polymerization. Typically, this approach requires reacting diols with diesters. Step-growth polymerizations are undesirable because of the heat needed to remove impurities such as generated water and the low molecular weights obtained.¹

To contrast step-growth, ring-opening polymerizations (ROP) has been used to synthesize a variety of polylactides and aliphatic polyesters. This method utilizes the enthalpically favored energy release of ring strain in order to drive the polymerization forward. One disadvantage to this approach is the lack of lactones available to do ROP, which significantly limits scope of polymers and properties that can be explored.^{7,8} One way to increase the range of options is to utilize the alternating co polymerization of epoxides and cyclic anhydrides. Ring opening co polymerization (ROCOP) is a method that utilizes catalysts that favor a truly alternating addition of two monomers.⁹ The possible mechanisms of this process are shown below in Scheme 2.1.

Scheme 2.1. Mechanistic Possibilities for Epoxide/Anhydride Copolymerization by use of (salen) MX Complexes^{a9}



^aCounterions are not shown for clarity. Salen = *N,N'*-bis(salicylidene)ethylenediamine.

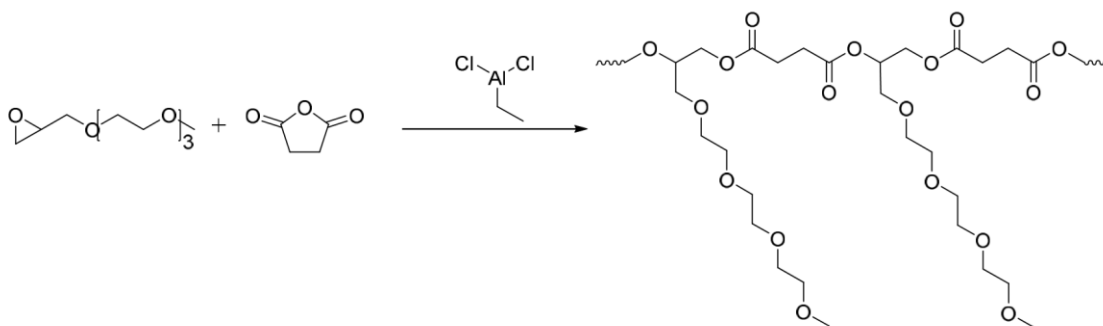
There are many advantages to using a ROCOP catalyst system to synthesize polyesters. To start, the scope of monomers that can be used is large, resulting in a wide array of polyesters and subsequent variation in their properties. Additionally, the use of a catalyst results in a polymerization that does not require an excessive amount of heat and significantly reduces the amount of undesirable side reactions.⁹ Finally, as will be explored below, ROCOP may be amenable to the incorporation of surface tethered cyclic monomers, which can be used for a grafting through mechanism to form polymer brushes.¹

2.1.2 Grafting Through

Grafting through is a mechanism by which the polymer can be tethered to the surface covalently, by presenting and incorporating a monomer that is bound to the surface. A previous group member, Alec Daye¹⁰ grew polymer brushes of succinic anhydride and a triethylene glycol-appended epoxide (ETEG, Scheme 2.2) with a grafting through

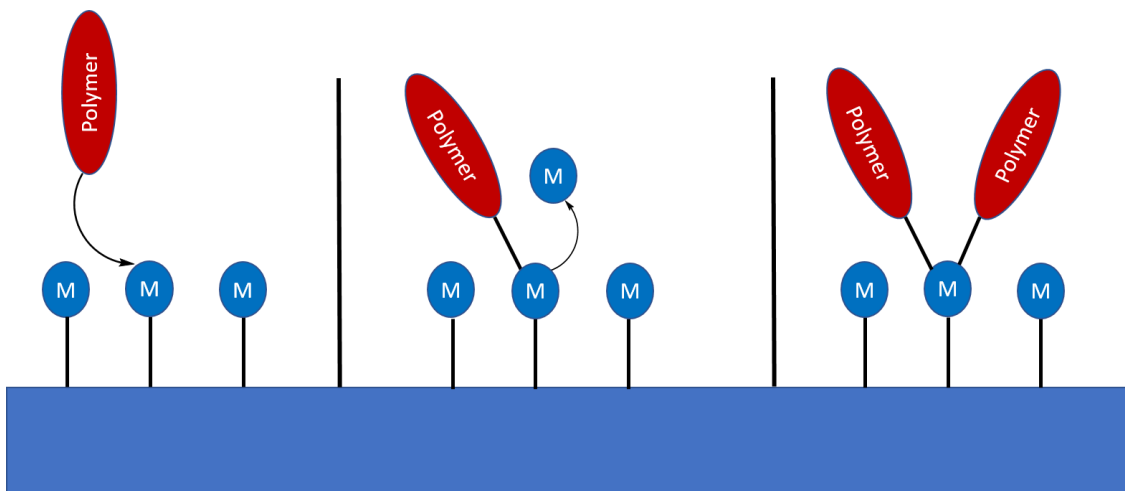
ROCOP approach using an epoxide functionalized surface. As will be discussed in more detail below, epoxysilanes are commercially available and there are many established procedures for tethering these to silicon wafers.¹¹⁻¹⁴ The grafting through mechanism is shown below in Scheme 2.3.

Scheme 2.2. ETEG and Succinic Anhydride Copolymerization



2.1.3 Goals

Scheme 2.3. Simplified Grafting Through mechanism



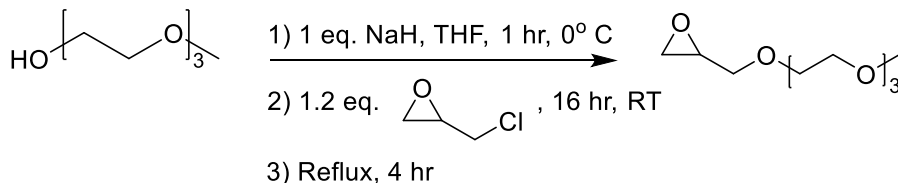
The goal of this work was to investigate the viability of creating Poly(maleic anhydride-co-ETEG) polymer brushes using a Cr(III) (salen) catalyst via a grafting through ROCOP mechanism. First, it had to be established that the copolymerization could be carried out in solution effectively. Second, it had to be established that the functionalization of the silicon wafers could be achieved with Glycidoxypropyltrimethoxysilane (GOPS). Finally, the two procedures had to be combined so that a polymer brush could be formed.

2.2: Results and Discussion

2.2.1 Synthesis of Epoxymethoxytriethylene Glycol

In order to impart a protein resistant moiety into the polyester, triethylene glycol branches needed to be incorporated into the polymer backbone. Oligoethylene glycol branches have previously been shown to resist proteins.¹⁵ This approach was adopted by Daye¹⁰ and involved coupling triethylene glycol monomethyl ether to epichlorohydrin (Scheme 2.4)

Scheme 2.4. Synthesis of ETEG.



Full details of the reaction are described in the experimental section. However, the reaction required vacuum filtration in order to obtain a completely pure product. The reaction gave yields ranging from 44% to 66%, which is slightly lower than the yield reported by Daye¹⁰. ¹H (Figure 2.1) and ¹³C NMR (Figure 2.2) of the distilled product indicated that the reaction was a success and that the final product was pure. It is important

to note that Peaks 1-3 in the ^1H NMR are used as diagnostic handles in the solution copolymerizations.

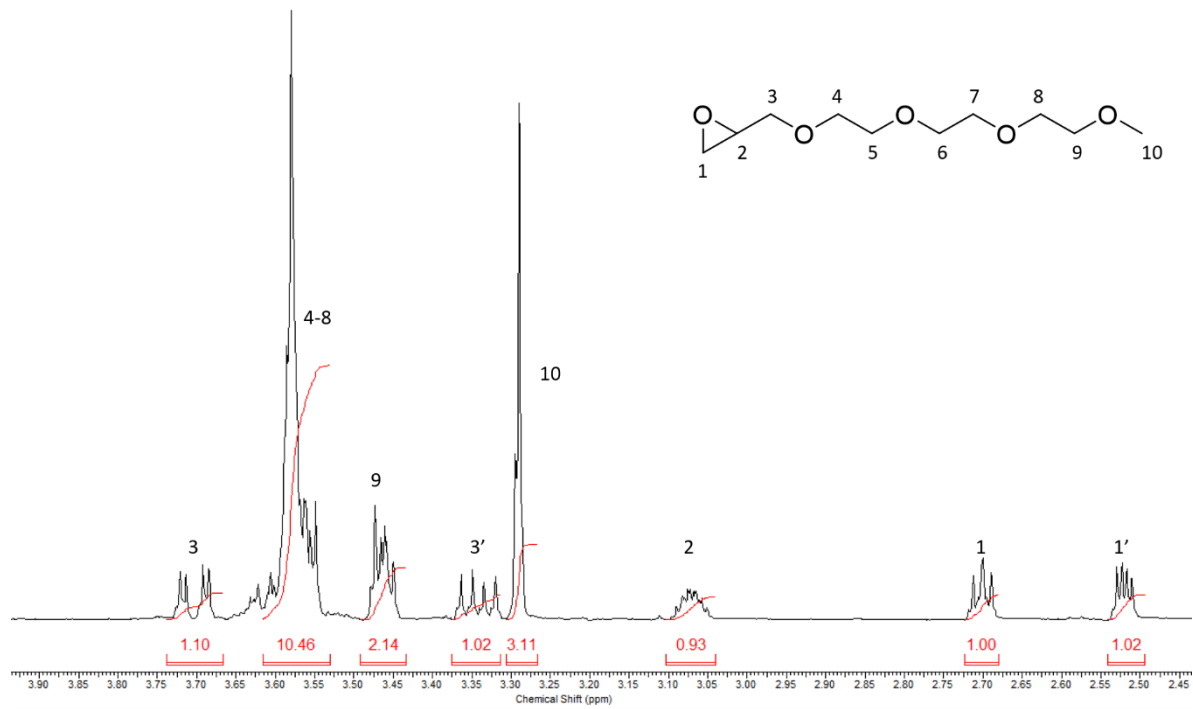


Figure 2.1. 400 MHz ^1H NMR of ETEG in CDCl_3

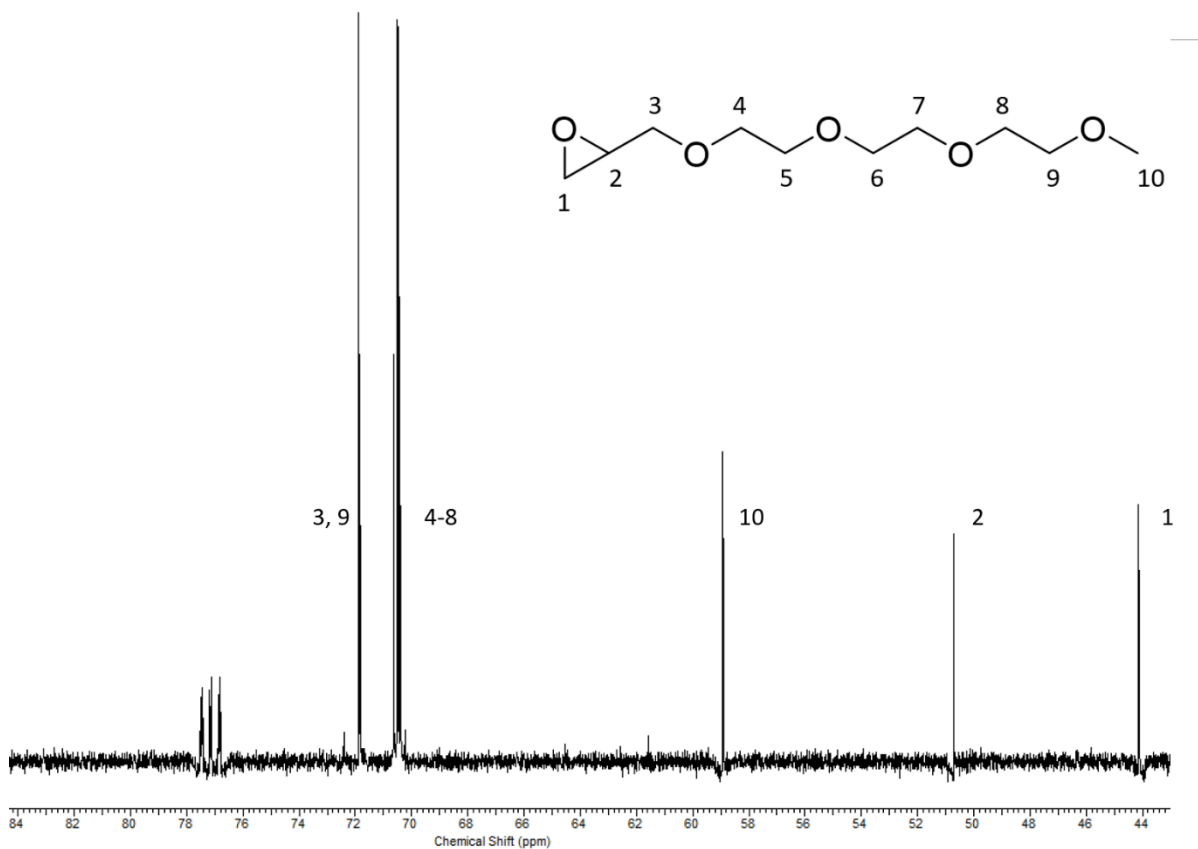


Figure 2.2. 400 MHz ¹³C NMR of ETEG in CDCl₃

2.2.2 Synthesis of Poly(MA-co-ETEG) and optimization

Copolymerization of maleic anhydride with a number of different epoxides was reported by Coates et al.⁹ In particular, a diethylene glycol appended epoxide was reported (Table 2.2 epoxide 4)¹⁷ The mild conditions, simple set up procedures, along with the high conversions and high MW's obtained were attractive for similar copolymerizations.

Scheme 2.6. ROCOP of MA and ETEG

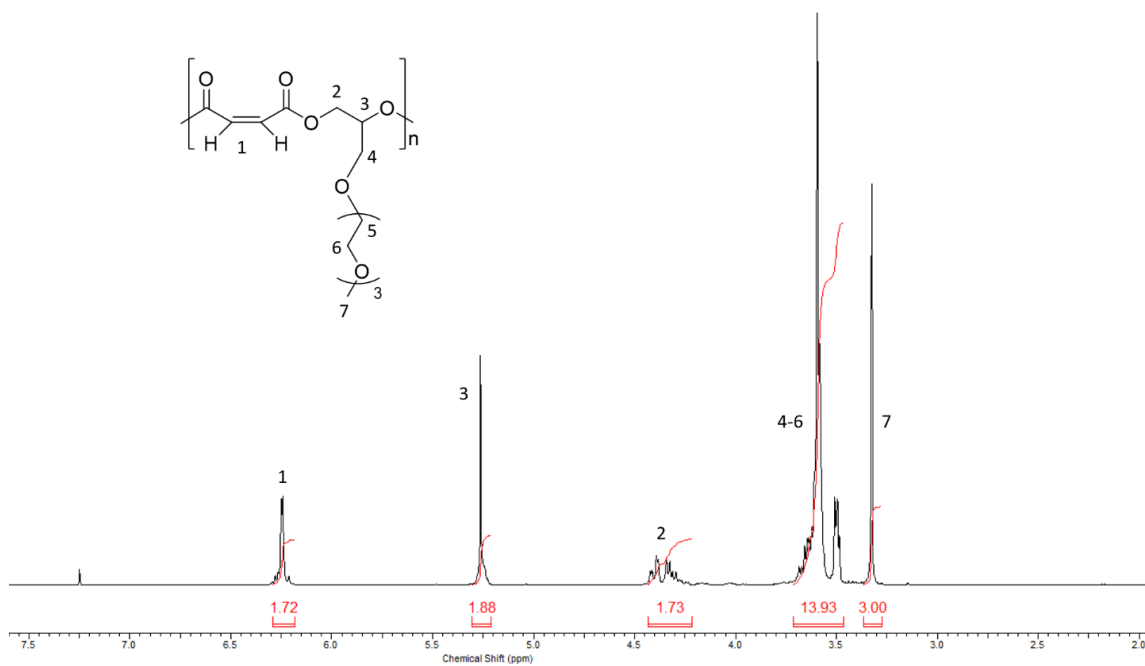
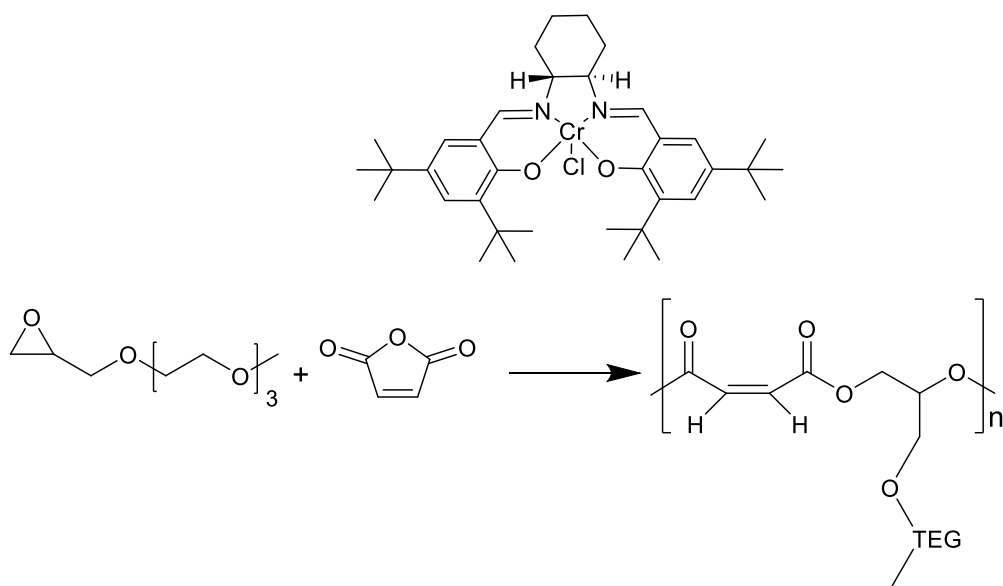


Figure 2.3. 400 MHz ¹H NMR of Poly(MA-co-ETEG) in CDCl₃

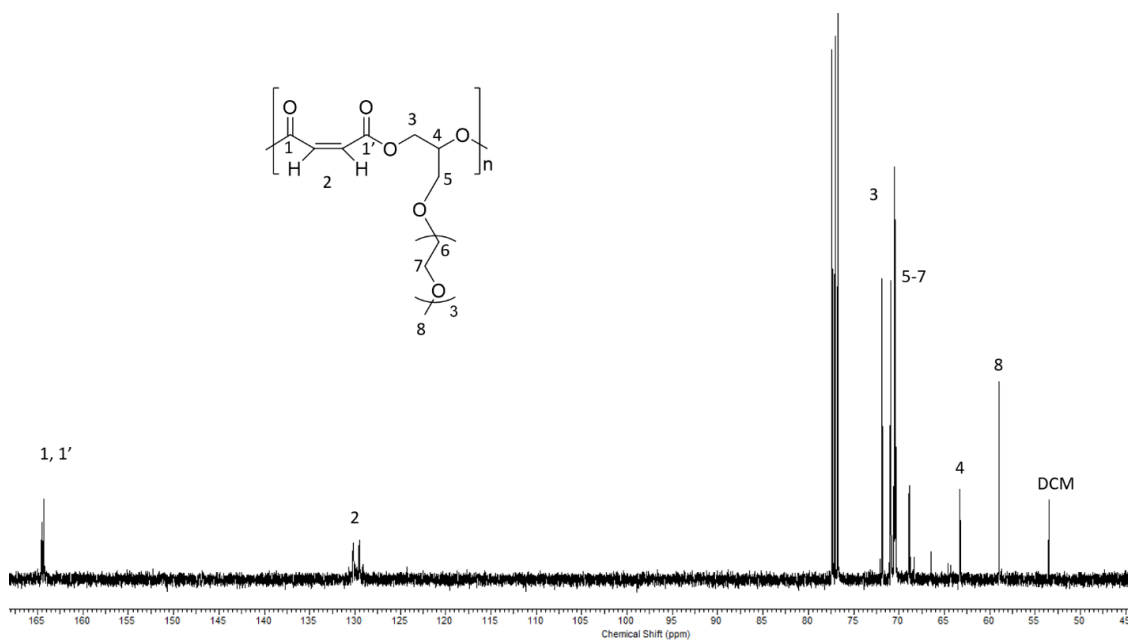


Figure 2.4. 400 MHz ^{13}C NMR of Poly(MA-co-ETEG) in CDCl_3

These assignments and integration match up very well with those as proposed by Coates.¹⁶ However, proton 3 has an integration that is a little higher than expected, most likely due to residual DCM. Additionally, there are some residual solvents in the ^{13}C as well. In the ^1H NMR, it must be noted that peaks (1-3) in ETEG (Figure 2.1) do not appear in the final polymer spectrum, indicating that all of the monomer has been removed. According to Coates¹⁶, the ether linkages in the pure homopolymer of ETEG appear at 78 ppm in ^{13}C but are overlapped in ^1H NMR from the ethylene glycol protons. The ^{13}C NMR of the polymer shows that there are no ether linkages appearing at 78 ppm, only the solvent signals at 77 ppm. This indicates the synthesis of a perfectly alternating copolymer.

In order to optimize yields and molecular weight, the polymerization was carried out at different times. This variation was explored because of an inability to match what the yield and molecular weight that Coates et al.¹⁶ had reported for a similar, diethylene glycol appended epoxide-monomer (Table 2.2 epoxide 4). The conditions of the polymerization were the same except a MW of only 2 kDa was obtained compared to their reported 33 kDa after the same amount of time. This polymerization was repeated and quenched at many different times. The data is shown below in Table 2.1. It was determined that the reaction was finished after 15 hours, giving a MW of 4.6 kDa. This MW is much lower than what Coates et al. had reported and is most likely due to a less active catalyst or trace impurities that deactivated the catalyst. In order to directly compare our results those reported by Coates et al., a polymerization was run using epichlorohydrin (entry 6) matching the conditions exactly to those reported in the literature. A polymer of undesirably low (9.1 kDa) molecular weight was obtained compared to a value of 25 kDa reported by Coates et al.¹⁶ Additionally, experiments were carried out in different solvents (THF and Toluene) in order to try to increase the MW. THF completely deactivated the catalyst and no polymer was obtained. Toluene gave similar MW compared to hexanes with the added benefit of dissolving everything much faster than hexanes. Use of hexanes resulted in initial phase separation between the hexanes and the liquid ETEG. Over an hour, the ETEG/hexanes mixed, possibly as the result of the presence of the growing copolymer. At any rate, toluene was used as the solvent in later grafting through experiments in order to ensure a homogenous solution. In addition to solvent choice, different stirring methods were also explored. Placing the vials in a sand bath with stir bars gave comparable MWs to running

the reactions on heated shaker tables. However, the ease of using shaker tables was determined to be a more effective stirring method during the grafting through experiments. However, given that Daye¹⁰ was able to show significant brush growth with smaller MW's via a grafting through mechanism using this polymer, it was determined at a MW of 4.6 kDa would be suitable to attempt grafting through ROCOP.

Table 2.1. Solution copolymerization of MA-ETEG using Cr(III)Cl (salen) as a catalyst.

| Entry | Time (h) | M _n (kDa) ^b | M _w (kDa) ^b | PDI ^b | Yield (%) |
|----------------|----------|-----------------------------------|-----------------------------------|------------------|-----------|
| 1 | 4 | 1.5 | 2 | 1.3 | 18 |
| 2 | 6 | 3.2 | 4.1 | 1.3 | 80 |
| 3 | 8 | 3.3 | 4.1 | 1.3 | 80 |
| 4 | 10 | 3.6 | 4.6 | 1.3 | 82 |
| 5 | 15 | 3.5 | 4.6 | 1.3 | 84 |
| 6 ^a | 15 | 8.8 | 9.1 | 1.0 | 76 |

Feed ratios MA:ETEG:Cat, 200:200:1, MA= 4 mmol in 1 mL of hexanes, Temperature= 45°C,
^a Epoxide= Epichlorohydrin. ^b Determined by GPC

Scheme 2.7. Copolymerization reported by Coates et al. with different epoxides using Cr(III)Cl (salen) as a catalyst.¹⁶

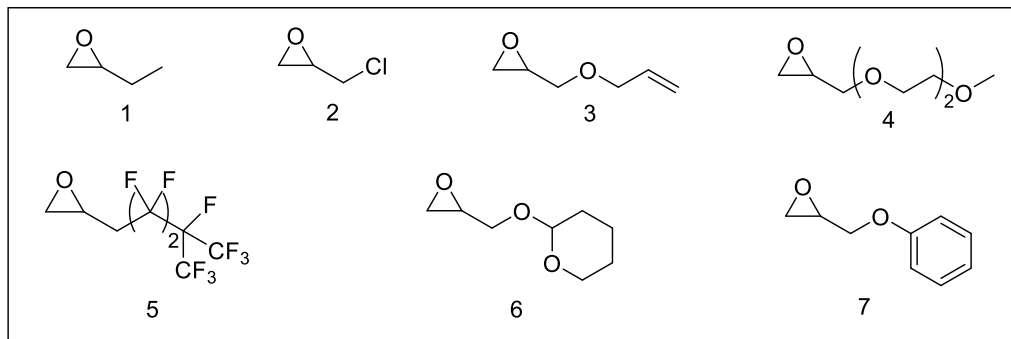


Table 2.2. Copolymerization reported by Coates et al. with different epoxides using Cr(III)Cl (salen) as a catalyst.¹⁶

| Epoxide | Time (h) | Conv (%) ^b | M _w (kDa) ^c | PDI ^c |
|---------|----------|-----------------------|-----------------------------------|------------------|
| 1 | 14 | 90 | 21 | 1.5 |
| 2 | 6 | 99 | 25 | 1.7 |
| 3 | 15 | 98 | 25 | 1.3 |
| 4 | 4 | 99 | 33 | 1.1 |
| 5 | 16 | 90 | 25 | 1.7 |
| 6 | 15 | 99 | 21 | 1.4 |
| 7 | 12 | 99 | 31 | 1.4 |

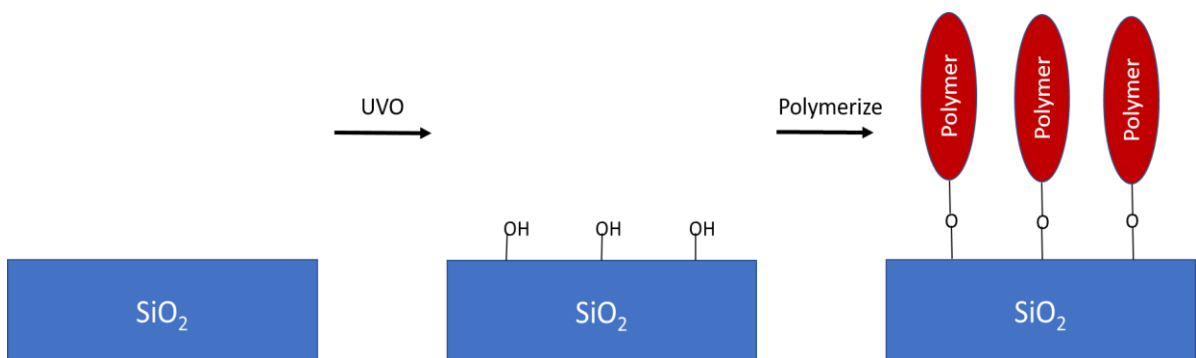
Feed Ratios MA:Epoxide:Cat= 200:200:1, MA= 4 mmol in 1 mL of hexanes, Temperature= 45 °C, ^b Determined by ¹H NMR spectroscopy of crude reaction. ^c Determined by GPC calibrated with polystyrene standards in CHCl₃ at 40°C.

2.2.3 Initial Surface Functionalization with Glycidoxypropyltrimethoxysilane (GOPS)

Several previous group members^{10, 17-19} have established that it is possible to graft poly(lactide) brushes from silicon wafers. Silicon wafers were subjected to ultraviolet ozonolysis (UVO), which oxidizes organic impurities on the surface and hydroxylates the native silicon, generating the silicon oxide. (Scheme 2.7) Daye¹⁰ also established that this treatment is the more effective method for generation of the silicon oxide, compared to

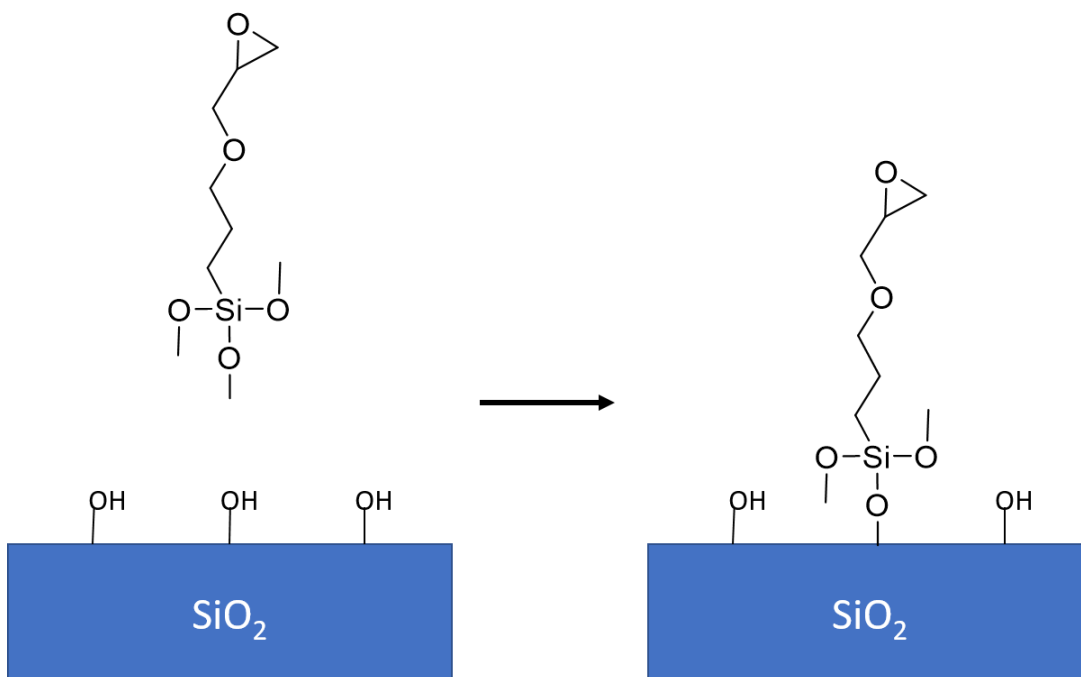
using Piranha washes to accomplish the same effect. The full cleaning process is described more fully in the experimental section.

Scheme 2.8. UVO treatment of silicon wafers for functionalization.



Once the wafers had their surfaces activated, they were immersed into a solution of glycidoxypropyltrimethoxysilane (GOPS) to prepare an epoxy-terminated surface. It was hoped that the growing polymer chain in solution would react the surface bound epoxy group and incorporate it into the polymer chain resulting in covalently attachment of the growing polymer chain to the surface and thus create a polymer brush. (Scheme 2.8)

Scheme 2.9. GOPS functionalization of silicon wafers.



Daye¹⁰ had established a procedure for GOPS functionalization that called for immersing the wafers in a 1% GOPS, 0.1% diisopropylethylamine (DIPEA) and toluene solution for 1 hour at room temperature. The DIPEA is used as a mild base that deprotonates the surface hydroxy group, which facilitates the addition of the GOPS to the surface.²⁰ The average thickness of the GOPS layer was determined to be 4.5 nm. by ellipsometry. These results indicate that there was significant multilayer formation and not monolayer formation. A fully extended GOPS monolayer on the surface should only be about 0.8 nm.²¹⁻²² The structure of this layer is unknown-it was too thin to obtain a viable infrared reflection spectrum, and it was decided that X-ray photoelectron spectroscopy would not give much useful information. Nevertheless, this method was used and the data is summarized below in Table 2.3. Thicknesses of 1.5-2.8 nm were obtained which is much

closer to the theoretical thickness of 0.8 nm, indicating that the functionalization was much closer to a SAM than what Daye¹⁰ was able to achieve. At this time, although no direct proof of the structure of the layer was available, it was hoped that this method of functionalization was sufficient to move forward to grafting through ROCOP experiments.

Table 2.3. Thicknesses of initial GOPS functionalization

| Wafer Number | Time (min) | Avg. thickness (nm) |
|--------------|------------|---------------------|
| 1 | 30 | 1.5 |
| 2 | 30 | 2.8 |
| 3 | 30 | 2.8 |
| 4 | 60 | 1.6 |

1% GOPS, 0.1% DIPEA in toluene, ^a Determined by ellipsometry

2.2.4 Grafting Through ROCOP at Different Molarities with a Cr(III)Cl (salen)

Catalyst

Satisfied that a proper method for surface functionalization of wafers with GOPS and a proper method for ROCOP had been established, the next step was to combine the two and attempt grafting through ROCOP. The wafers were functionalized in the previously described method. However, it should be noted that for the grafting through portion, these polymerizations were carried out in toluene and done on the shaker to ensure that a homogenous solution was present and that the solution was effectively stirred over the wafer. The molarities were calculated with respect to the amount of MA and toluene added to the vials. A more complete description of the methods used can be found in the experimental section. The data obtained using different molarities is summarized below in Table 2.4.

Table 2.4. Average Polymer Growth of wafers at different molarities of solution.^a

| (M) ^b | Wafer number | Time (h) | Avg. Growth (nm) ^c |
|------------------|--------------|----------|-------------------------------|
| 0.1 | 1 | 48 | 0.6 |
| 0.1 | 2 | 48 | 0.0 |
| 1 | 1 | 34 | 0.0 |
| 1 | 2 | 1 week | 1.8 |
| 4 | 1 | 18 | 0.0 |
| 8 | 1 | 15 | 3.6 |

^a Wafer functionalization 1% GOPS, 0.1% DIPEA in toluene, RT, 1 hour, 1 wafer kept as reference wafer, Polymerization feed ratio MA:ETEG:Cat 200:200:1, 45 °C on shaker table, ^b Calculated with respect to [MA], ^c Calculated by thickness (nm) of reference wafer - thickness (nm) after polymerization

The data above indicates a failure to graft through at different concentrations. The data at 1 M for 1 week was promising with a polymer growth of 1.8 nm. but this growth was unable to be reproduced. The growth at 8 M also looked promising but the growth was most likely due to physisorbed material as this reaction completely buried the wafer in polymer. In all of these reactions, the polymers obtained in solution and had reasonable MWs. With it being established that the ROCOP was not the issue, other attempts were made to explore the GOPS functionalization as the source of the error.

2.2.5 Further Attempts at GOPS Functionalization

Given that the most likely reason that the grafting through ROCOP failed was due to problems regarding the GOPS functionalization of the wafers, alternative strategies were explored. These include using a higher concentration of GOPS in the functionalization solution, using a higher temperature and running the functionalization reaction for a longer period of time.²⁰ Table 2.5 shows the effect of increasing reaction time on the thickness of the functionalized layer. No meaningful change in thickness was obtained.

Table 2.5. Higher Temperature GOPS functionalization.^a

| Wafer number | Time (h) | Avg. thickness (nm) ^b |
|--------------|----------|----------------------------------|
| 1 | 18 | 1.4 |
| 2 | 48 | 2.2 |
| 3 | 1 week | 1.9 |

^aWafer Functionalization Toluene:GOPS:DIPEA 100:30:1, 85 °C, ^b Determined by ellipsometry

The higher concentration of GOPS, higher temperatures and longer times failed to produce any difference in thickness compared to the previous method of functionalization. The next attempt was stir the solutions during the functionalization reaction. Previously, the wafers simply sat in the solution and there was no attempt made to stir the solutions. To achieve this agitation, the wafers in the solution were sealed, and removed from the glove box and placed on a shaker table. Additionally, the temperature was increased to 50°C in order to give the GOPS the best possible chance to bond with the surface. The results of this are presented in Table 2.6 below.

Table 2.6. Increased Stirring functionalization of GOPS.^a

| Wafer Number | Time (h) | Avg. Thickness (nm) ^b |
|--------------|----------|----------------------------------|
| 1 | 0.5 | 2.3 |
| 2 | 2 | 2.8 |
| 3 | 24 | 3 |
| 4 | 40 | 2.3 |

^a Wafer Functionalization 1% GOPS, 0.1% DIPEA in toluene, 50°C in shaker table, ^b Determined by ellipsometry

Generally, these thicknesses are thicker compared to the unstirred functionalization reactions. Additionally, there seems to be a trend of thickness increasing over time, however this was not observed with wafer 4, perhaps due to a contaminated wafer. Assuming that there was a monolayer of GOPS on the surface, one of these wafers was

grafted through ROCOP in the normal conditions mentioned above at 4 M polymer solution and failed to produce any thickness change after the polymerization. Still assuming that a GOPS monolayer was present on the surface, the next attempt was to try and functionalize the surface to match the multilayer thickness observed by Daye.¹⁰

Daye¹⁰ was able to obtain multilayers of GOPS even after a short time of functionalization time. The data below are the results of those past experiments. (Table 2.7)

Table 2.7. Film Thickness of GOPS treated wafers reported by Daye.¹⁰

| Time | Cond. 1 ^{a,c} Thickness (nm) | Cond. 2 ^{b,c} Thickness (nm) |
|------|---------------------------------------|---------------------------------------|
| 24 h | 10.6 | 10.1 |
| | 6.93 | 10.1 |
| | 8.79 | 10.3 |
| 18 h | 6.72 | 9.70 |
| | 6.78 | 9.91 |
| | 6.76 | 8.93 |
| 3 h | 3.27 | 6.28 |
| | 2.73 | 6.54 |
| | 3.14 | 6.57 |
| 1 h | 2.74 | 3.43 |
| | 3.09 | 3.45 |
| | 2.78 | 3.42 |
| 30 m | | 1.22 |
| | | 0.99 |
| | | 0.80 |
| 10 m | | 0.40 |
| | | 0.49 |
| | | 0.53 |

^a1% GOPS in toluene. ^b1% GOPS and 0.1% diisopropylethylamine in toluene. ^cDetermined by ellipsometry.

The fact that Daye¹⁰ could grow multilayers in such a short amount of time is most likely due to trace amounts of impurities present, such as water. Water has been shown to induce multilayer formation in silanes.²⁰ In order to replicate this data, the GOPS

functionalization was done outside of the glove box, taking no precautions to avoid contaminants or water. The results of this are shown below in Table 2.8.

Table 2.8. GOPS functionalization outside of the glove box.

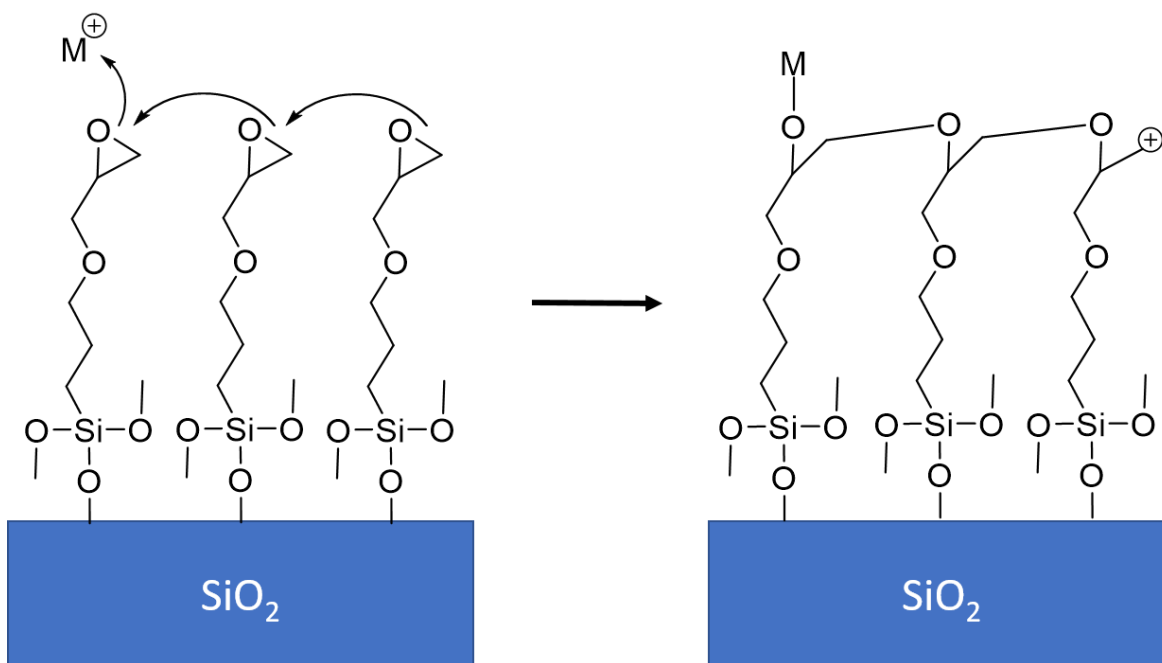
| Wafer Number | Avg. Thickness (nm) ^a |
|--------------|----------------------------------|
| 1 | 1.3 |
| 2 | 1.8 |
| 3 | 1.4 |

Wafer Functionalization 1% GOPS, 0.1% DIPEA in toluene, 50 °C in shaker table for 2 hours, ^a Determined by ellipsometry

The average thickness was very consistent with monolayer formation and with previous experiments conducted. However, the results do not match at all with those obtained by Daye.¹⁰ The thickness difference is quite large of 4-5 nm compared to 1-3 nm obtained under the same conditions.

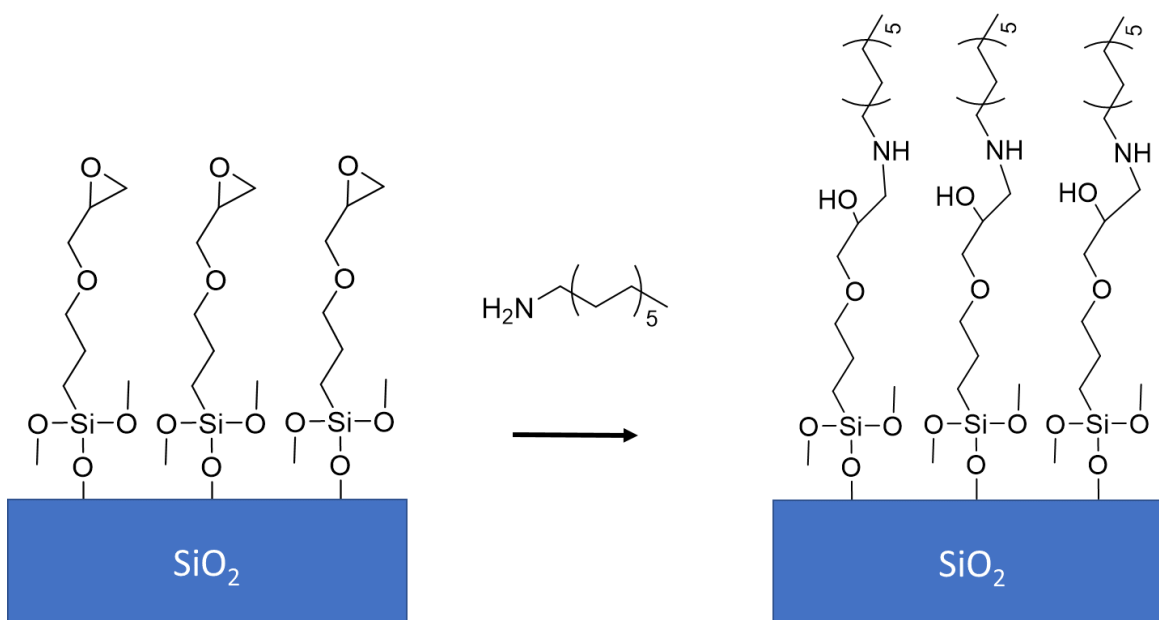
It is possible that the GOPS monolayer is becoming deactivated and thus unable to form multilayers. This could also potentially explain the reason why the grafting through ROCOP had failed. A GOPS monolayer can react with itself to form poly(ethylene glycol) across the surface and render it chemically inert as shown in Scheme 2.9 below. However, Yeager et al. attempted to do just this transformation using organothiol SAM on gold, and did not report any successful conversions.¹¹

Scheme 2.10. GOPS deactivation mechanism



It has been reported in the literature that primary amines can react with the epoxide on GOPS and form a covalent bond with the surface.²³ In order to test the reactivity of GOPS, a functionalized wafer was immersed in a 2 mM solution of dodecylamine in ethanol for 2.5 hours. Dodecylamine was chosen because of the reactive primary amine on one end, and for its long tail group, which should result in a measurable increase in ellipsometrically-determined thickness after successful functionalization. Specifically, in a fully extended conformation, the thickness of the functionalized wafers shown in Scheme 2.10 below should be ca. 1.8 nm. thicker. However, this functionalization did not yield any significant thickness increase compared to the reference wafer. This result indicates that the epoxide groups are unreactive by transformation due to some side reaction.

Scheme 2.11. GOPS-Amine reaction



2.2.6 ((Et)AlCl₂) Grafting Through ROCOP

In order to further examine this system, Daye's work¹⁰ was repeated as closely as possible. Table 2.9 below summarizes the results of the GOPS functionalization and the results of the ROCOP using an ((Et)AlCl₂) catalyst system. For the GOPS functionalization, Daye¹⁰ obtained very consistent thicknesses of around 5.2 nm with one exception being wafer 1 of 7.7 nm. These results are consistent but again represent a multilayer formation. However, the brush growth is very different from wafer to wafer, ranging from 3.3 to 13.9 nm of growth. These results indicate that impurities or catalyst deactivation is playing a role in preventing uniform brushes to form. Nevertheless, despite the nonuniformity, every wafer showed a thickness increase of the grafted layer upon exposure to the monomer and catalyst. Replication of these experiment had two purposes. First, it could show that chemically active GOPS was on the surface and could initiate brush growth. Second, if growth was observed,

then the reason that previous experiments failed could be traced to the use of the Cr(III)Cl (salen) catalyst as opposed to the use of ((Et)AlCl₂).

Table 2.9. GOPS Functionalization and grafting through ROCOP reported by Daye.¹⁰

| | Wafer 1 | Wafer 2 | Wafer 3 | Wafer 4 | Wafer 5 | Wafer 6 |
|--|---------|---------|---------|---------|---------|---------|
| Average GOPS (nm) ^a | 7.70 | 5.23 | 5.31 | 5.24 | 5.20 | 5.43 |
| Grafting Through (nm) ^b | 11.0 | 10.4 | 12.6 | 9.45 | 14.5 | 19.3 |
| Calculated Brush/Film Layer ^c | 3.25 | 5.12 | 7.32 | 4.21 | 9.31 | 13.9 |

^a 100:30:1 Toluene:GOPS:DIPEA at 85 °C for 48 hours. ^bGrafting Through GOPS. [Succinic Anhydride] = 0.1 M. Polymerization feed ratios SA:ETEG:Cat 100:100:1, In 1,4-dioxane at 70 °C for 60 hours. ^c Calculated by grafting through minus GOPS

There were a few differences between the previously reported experiments and the experiments reported here. First, the GOPS functionalization was changed to a 1% GOPS, 0.1% DIPEA in toluene solution at 50 °C for 1 hour on the shaker table. This method had currently been established as the superior option. Second, the solvent for the grafting through ROCOP was changed from 1,4-dioxane to toluene. This was done because the ((Et)AlCl₂) was in a stock solution of toluene in the freezer. Third, the time was decreased slightly from 60 hours to 48 hours. Additionally, this experiment was repeated and the stirring method changed from a heated Al block to heating in an oil bath to ensure better mixing and encourage more growth. The results of the new experiments are listed below in Table 2.10. The results below indicate that the surface was chemically active enough to form small brushes with the catalyst.

Table 2.10. Grafting Through ROCOP with ((Et)AlCl₂) catalyst.^a

| Wafer Number | Avg. Growth (nm) ^{b,c} | Heating method |
|--------------|---------------------------------|----------------|
| 1 | 1.7 | Al Block |
| 2 | 1.2 | Al Block |
| 1 | 1.6 | Oil Bath |
| 2 | 3.0 | Oil Bath |

^aWafer Functionalization 1% GOPS, 0.1% DIPEA in toluene, 50°C in shaker table for 2 hours. MA= 0.1 M, Polymerization feed ratio MA:ETEG:Cat 100:100:1 in toluene, 70°C, 48 hours. ^b Calculated by thickness after polymerization – thickness of reference wafer. ^c Determined by ellipsometry.

2.3 Conclusions

2.3.1 Solution Polymerization

ETEG was synthesized according to Daye's procedure.¹⁰ The spectroscopic data matched what was reported however, the yield was slightly lower than expected. With respect to the ROCOP, the ¹H and ¹³C NMR data matched very well with what Coates et al. had reported. However, the MWs obtained were significantly lower than expected. It has been observed by Feng²⁵ that as the length of the epoxide moiety is increased, the MW obtained decreases. The epoxide used was one ethylene glycol unit longer than the one reported by Coates, which might explain the lower weights.

2.3.2 Surface Modification with GOPS

Surface attachment of GOPS proved to be very reproducible and occurred under many different conditions. However, forming multilayers of GOPS was not able to be achieved perhaps due to contaminants of some sort existing in Daye's experiments.¹⁰ The results obtained clearly indicate that something much closer to resembling a monolayer were obtained. The experiments using the ((Et)AlCl₂) catalyst showed enough growth, which

indicates that the GOPS on the surface is at least partially chemically reactive. The brush thicknesses obtained were not as reported previously, however, this result indicates that some of the epoxides on the surface are being rendered chemically inert, decreasing obtained thickness.

2.3.3 Grafting Through ROCOP

The grafting through ROCOP experiments using the Cr(III) (salen) catalyst failed under multiple different conditions as evidence by lack of any appreciably increased layer thickness. As stated above, the thickness increase due to the ((Et)AlCl₂) catalyst indicates that GOPS was reactive enough in order to produce a thickness increase. With GOPS functionalization not being the deciding issue, the only other explanation is that the failure was due to the use of Cr(III) (salen) as a catalyst. Given the size of the Cr(III) (salen) catalyst, there is concern that this catalyst is too big to coordinate with an epoxide group, open it and then have the carboxy anion attack the coordinated epoxide within the confines of a monolayer on the surface. On the other hand, the ((Et)AlCl₂) catalyst is much smaller, and thus is able to react with the GOPS monolayer more effectively. This can be seen because the ((Et)AlCl₂) catalyst showed modest growth. However, the growth obtained was much less than what Daye¹⁰ obtained. Another possible explanation for this is that Daye's GOPS functionalization formed multilayers,¹⁰ which created an environment on the surface in which epoxides had more freedom to move around and their distance from the surface was not uniform. This nonuniformity increases the chances that the catalyst was able to find an epoxide and start growing a polymer brush, thus giving thicker brushes. The relative sterics of the functionalized GOPS layer provides a reasonable explanation as to why thinner

brushes were obtained when replicating previous experiments and why the use of the very large Cr(III) (salen) catalyst failed as well.

2.4 Experimental

2.4.1 Solvents and Glassware

THF and Toluene were acquired from an in-house solvent purification system and moved into the glove box where they were stored under molecular sieves. Hexane was stored under sieves in the glovebox. All other solvents were used as received. All glassware was washed with acetone, soap and water and then acetone again. All glassware was either flame dried or oven dried and moved into the glovebox while still hot.

2.4.2 Instrumentation

Polymers were characterized by NMR with a 400 MHz Varian Inova 400 spectrometer at room temperature. Polymer molecular weights were obtained with a PL-GPC 50 Plus using a Jordi-Gel DVB 1000A column with a refractive index detector. The solvent used was distilled THF at 1 mL/min at 35°C. Near monodisperse polystyrene standards were obtained from Fluka ranging from 250-70000 Da. Wafer thickness was determined by use of Variable-Angle Spectroscopic Ellipsometry (VASE, J.A. Woollam, USA). The model used to fit was a simple Cauchy layer followed by a SiO₂ layer and then a Si layer. Angle of measurement was 70°.

2.4.3 ETEG Synthesis^{10,24}

A two-necked round bottom flask was loaded with 6.92 grams of NaH that was 60% oil and purged with nitrogen. The flask was filled with 50 mL of hexanes with a syringe and stirred. The stirring was stopped, allowing the NaH to settle and the hexanes were removed with a syringe. This process was repeated two more times. After the last wash, 30 mL of THF was added to the flask and the flask was placed in an ice bath where 25 mL of triethylene glycol monomethyl ether was added slowly with a pressure equalizing addition funnel. The reaction was allowed to react for two hours at room temperature. The flask was placed in an ice bath again and 25 mL of distilled epichlorohydrin was added slowly with a pressure equalizing addition funnel. The reaction was then allowed to react overnight at room temperature. The flask was equipped with a reflux condenser and allowed to reflux for 4.5 hours. The reaction was then filtered using two Whatman filter papers and then the salt byproduct was washed three times with THF. The salt was then washed with water and extracted three times with 30 mL of chloroform. The chloroform layers were combined with the THF layer and then all solvents were removed via rotatory evaporation. The product was fractionally vacuum distilled to give a clear, slightly viscous liquid at 66% yield. ¹H NMR (CDCl₃, 400 MHz): δ 2.52 (dd, 1H), 2.70 (t, 1H), 3.07 (m, 1H), 3.29 (s, 3H), 3.35 (dd, 1H), 3.46 (m, 2H), 3.57 (m, 10H), 3.70 (dd, 1H). ¹³C NMR (CDCl₃, 125 MHz): δ 44.0, 50.6, 58.8, 70.3, 70.4, 70.4, 70.4, 70.6, 71.8, 71.8

2.4.4 Solution Copolymerization

A typical polymerization was as follows: inside a dinitrogen filled glovebox, 0.02 mmol of (R,R)-N,N'-Bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexanediaminochromium(III) chloride (Cr(III)Cl(salen)), 4.0 mmol of sublimed maleic anhydride, 4.0 mmol of ETEG, ~ 1 mL of hexanes and a stir bar were added to a 20 mL scintillation vial. A screw cap with a teflon liner was then added to the vial, and the vial was sealed with electrical tape. The solids in the vial were then allowed to dissolve and then the vial was placed in an oil bath at 45 °C overnight while stirring using a magnetic stir bar. The vial was opened and a minimal amount of DCM was added until all the polymer was dissolved. The polymer was then precipitated in about 50 mL of diethyl ether. The liquid was decanted off into centrifuge tubes and spun at 2500 RPM for 10 minutes. The liquid was poured off and the polymer at the bottom of the centrifuge tubes was combined. The solvent was removed with rotary evaporation followed by exposure to high vacuum. The polymer was characterized with a 400 MHz ¹H NMR and ¹³C NMR and by gel permeation chromatography using a refractive index detector in THF. ¹H NMR (CDCl₃, 400 MHz): δ 3.33 (s, 3H), 3.60 (m, 14H), 4.37 (m, 2H), 5.25 (s, 1H), 6.24 (s, 2H). ¹³C NMR (CDCl₃, 125 MHz): δ 164.5, 164.3, 130.3, 129.5, 71.6, 71.2, 70.6, 70.4, 68.8, 63.3, 59.1.

2.4.5 Wafer Preparation

Mechanical grade silicon wafers were obtained from Silicon Valley Microelectronics with an orientation of 100°, thickness of 0.5 mm, and diameter of 100 mm and were cut into 1.5 to 2.0 cm squares. The wafers were then cleaned and hydroxylated

with UV-Ozonolysis treatment for 30 minutes. The wafers were immediately brought into a dinitrogen-filled glovebox and immersed in a 1% glycidoxypropyltrimethoxysilane (GOPS) and 0.1% diisopropylethylamine solution in toluene. The wafers were all immersed in the same solution and allowed to react for a given time. When doing the functionalization on the shaker table, the wafers were placed into vials and about 10 mL of the GOPS solution was placed in each vial, sealed and then removed from the glove box and placed on the shaker table at 250 RPM at the given time and temperature. The wafers were then sonicated in toluene for 3-5 minutes, rinsed with methanol and dried under a stream of nitrogen gas.

2.4.6 Grafting Through ROCOP

The functionalized wafers were then brought back into the dry box and placed in 20 mL scintillation vials. The desired quantities of maleic anhydride, ETEG and catalyst mentioned above were added vials along with the desired amount of toluene in order to give the correct molarity of the reaction with respect to the concentration of maleic anhydride. The vials were equipped with a cap containing Teflon liner, sealed with electrical tape and removed from the glove box and placed on the shaker table at 45 °C at 250 RPM. After the reaction was completed, DCM was added to dissolve the physisorbed polymer off the wafers as needed. The wafers were sonicated in toluene for 3-5 minutes, and blown dry with a stream of nitrogen gas. The wafers were then analyzed with ellipsometry. In the case of the ((Et)AlCl₂) catalyst reactions, 20 mL scintillation vials were equipped with a small glass circle that allowed for a small stir bar to rotate unhindered in the center and over top of this, a smaller piece of wire mesh was placed that covered the

bottom of the vial. The functionalized wafers were brought back into the dry box and placed on top of the mesh. Then from a stock solution of 0.1 M maleic anhydride and ETEG in toluene was added to the vial in the desired amount. Then a stock solution of $((\text{Et})\text{AlCl}_2)$ catalyst in toluene was diluted down and added to the vial. Teflon liners were added to the vials and sealed with electrical tape. These were then either placed in the Al block or an oil bath at the temperature indicated. After the reaction was completed, the wafers were removed, sonicated in toluene, washed in toluene and dried under a stream of nitrogen gas followed by ellipsometry analysis.

2.5 References

- (1) Odian, G. Principles of Polymerization, 4th ed.; John Wiley & Sons: Hoboken, NJ, 2004.
- (2) Hillmyer, M. A.; Tolman, W. B.; Aliphatic polyester block polymers: Renewable, degradable, and sustainable. *Acc. Chem. Res.* **2014**, 47, 2390–2396.
- (3) Gross, R. A.; Kalra, B.; Biodegradable Polymers for the Environment. *Science* **2002**, 297, 803–807.
- (4) Amass, W.; Amass, A.; Tighe, B.; A Review of Biodegradable Polymers: Uses, Current Developments in the Synthesis and Characterization of Biodegradable Polyesters, Blends of Biodegradable Polymers and Recent Advances in Biodegradation Studies. *Polym. Int.* **1998**, 47, 89–144.
- (5) Olson, D. A.; Gratton, S. E. A.; DeSimone, J. M.; Sheares, V. V.; Amorphous Linear Aliphatic Polyesters for the Facile Preparation of Tunable Rapidly Degrading Elastomeric Devices and Delivery Vectors. *J. Am. Chem. Soc.* **2006**, 128, 13625–13633.
- (6) Nuyken, O.; Pask, S. D.; Ring-Opening Polymerization-An Introductory Review *Polymers.* **2013**, 5 (2), 361–403.
- (7) Lecomte, P.; Jérôme, C. Recent Developments in Ring-Opening Polymerization of Lactones. In *Synthetic Biodegradable Polymers*; Rieger, B., Künkel, A., Coates, G. W., Reichardt, R., Dinjus, E., Zevaco, A. T., Eds., *Advances in Polymer Science*, 245, **2012**, 173–217

- (8) Paul, S.; Zhu, Y.; Romain, C.; Brooks, R.; Saini, P. K.; Williams, C.K.; Ring-Opening Copolymerization (ROCOP): Synthesis and Properties of Polyesters and Polycarbonates. *Chem. Commun.* **2015**, 51, 6459– 6479.
- (9) Longo, J.M.; Sanford, M.J.; Coates, G.W.; Ring-Opening Copolymerization of Epoxides and Cyclic Anhydrides with Discrete Metal Complexes: Structure–Property Relationships. *J. Am. Chem. Soc.* **2016**, 116, 15167-15297
- (10) Daye, A.J.; Synthesis of Protein-Resistant, Biodegradable Polymer Brushes, M.S. Dissertation, North Carolina State Univ., **2016**.
- (11) Yeager, L.J.; Amirsakis, D.G.; Newman, E.; Garell, R.L.; Synthesis of Epoxy-Terminated Dialkyl Disulfides for Polymerizable Self-Assembled Monolayers. *Tet. Let.* **1998**, 39, 8409-8412.
- (12) Stamm, M. *Polymer Surfaces and Interfaces*, 1st. Ed.; Springer-VBH: Berlin, Germany, **2008**.
- (13) Brittain, W. J.; Minko, S. J.; A Structural Definition of Polymer Brushes. *Polym. Sci. Part A Polym. Chem.* **2007**, 45, 3505– 3512.
- (14) Henze, M.; Mädge, D.; Prucker, O.; Rühle, J.; “Grafting Through”: Mechanistic Aspects of Radical Polymerization Reactions with Surface-Attached Monomers. *Macromolecules*, **2014**, 47, 2929-2937.
- (15) Edmondson, S.; Osborne, V.L.V.; Huck, W.T.; Polymer Brushes via Surface-initiated Polymerizations *Chem. Soc. Rev.* **2004**, 33, 14–22.

- (16) Haskard, C.A.; Li-Chan, E.C.Y.; Hydrophobicity of Bovine Serum Albumin and Ovalbumin Determined Using Uncharged (PRODAN) and Anionic (ANS⁻) Fluorescent Probes. *J. Agric. Food Chem.* **1998**, 46, 2671–2677.
- (17) DiCiccio, A.M.; Coates, G.W.; Ring-Opening Copolymerization of Maleic Anhydride with Epoxides: A Chain-Growth Approach to Unsaturated Polyesters. *J. Am. Chem. Soc.*, **2011**, 133, 10724-10727.
- (18) Hu, X. Investigation of Growth and Degradation Behaviors of Biodegradable Polyester Brushes and Their Applications. Ph.D. Dissertation, North Carolina State Univ., **2013**.
- (19) Xu, L.; Gorman, C.B.; Poly(Lactic Acid) Brushes Grow Longer at Lower Temperatures. *J. Polym. Sci. Part A Polym. Chem.* **2010**, 48, 3362–3367.
- (20) Chen, X.; Ring Opening Copolymerization of Succinic Anhydride. *Chinese J. Polym. Sci.* **1997**, 15, 262–272
- (21) Wong, A.K.Y.; Krull, U.J.; Enhanced Osteoblast Adhesion to Epoxide-Functionalized Surfaces. *Anal. Bio. Chem.* **2005**, 838, 187-200.
- (22) Tsutruk V.V.; Luzinov I.; Julthongpipit D; Tribological Behavior of Grafted Polymer Gel Nanocoatings. *Langmuir*, **1999**, 15,3029–3032.
- (23) Luzinov I.; Julthongpipit D.; Liebmann-Vinson A.; Cregger T.; Foster M.D., Tsukruk V.V.; Epoxy-Terminated Self-Assembled Monolayers: Molecular Glues for Polymer Layers. *Langmuir*, **2000**, 16, 504–516.

- (24) Zhou, W.; Burke, P.J.; Versatile bottom-up synthesis of tethered bilayer lipid membranes on nanoelectronic biosensor devices. *ACS Appl. Mater. Interfaces*, **2017**, pending
- (25) Jungk, S. J.; Moore, J. A.; Gandour, R. D.; Efficient Synthesis of C-Pivot Lariat Ethers. 2-(Alkoxy methyl)-1,4,7,10,13,16-hexaoxa-cyclooctadecanes. *J. Org. Chem.* **1983**, 48, 1116–1120.
- (26) Feng, L.; Liu, Y.; Hao, J.; Li, X.; Xiong, C.; Deng, X.; Synthesis and Properties of Novel Thermoresponsive Polyesters with Oligo(ethylene glycol) Pendent Chains. *Macromol. Chem. Phys.* **2011**, 2626-2632.

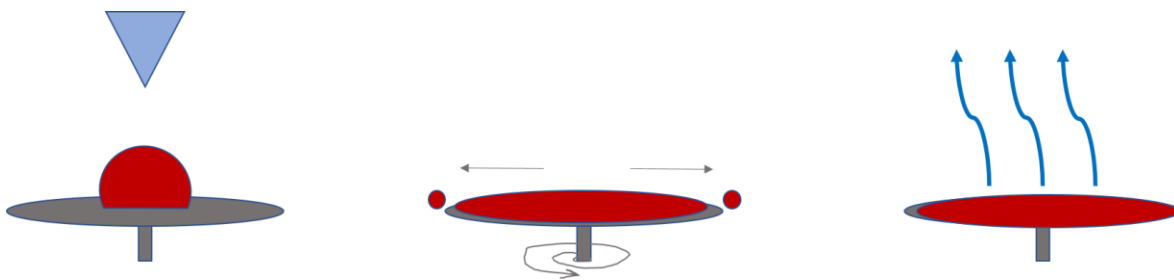
Chapter 3 Spin Coating Polyestersers onto Surfaces: An Alternative Approach to Brushes

3.1 Introduction

3.1.1 Spin Coating

After it had been established that the formation of polymer brushes with a grafting through ROCOP mechanism had failed using Cr(III)Cl (salen) as a catalyst, alternative methods were explored. Although, the ROCOP had proven to be very robust and produce polymer reliably, it was determined that another way of anchoring this polymer to a surface was needed. There are many ways of producing thin films, but one of the easiest ways is by spin coating. Spin coating involves placing a wafer on a disk that will spin it at a controlled rate for a controlled amount of time. A solution of the polymer is deposited on the surface of the clean wafer and then spun at the desired rate and time. As the wafer spins, excess polymer is spun off the surface, giving a uniform coating. After the wafers have a film on the surface, the surface is annealed so that excess solvent evaporates and the polymer can settle. A description of spin coating is pictured below in Scheme 3.1.

Scheme 3.1. Spin Coating onto Wafers



Spin coating is much easier, faster and more controllable compared to forming polymer brushes. Formation of polymer brushes tends to be less reproducible, and it is

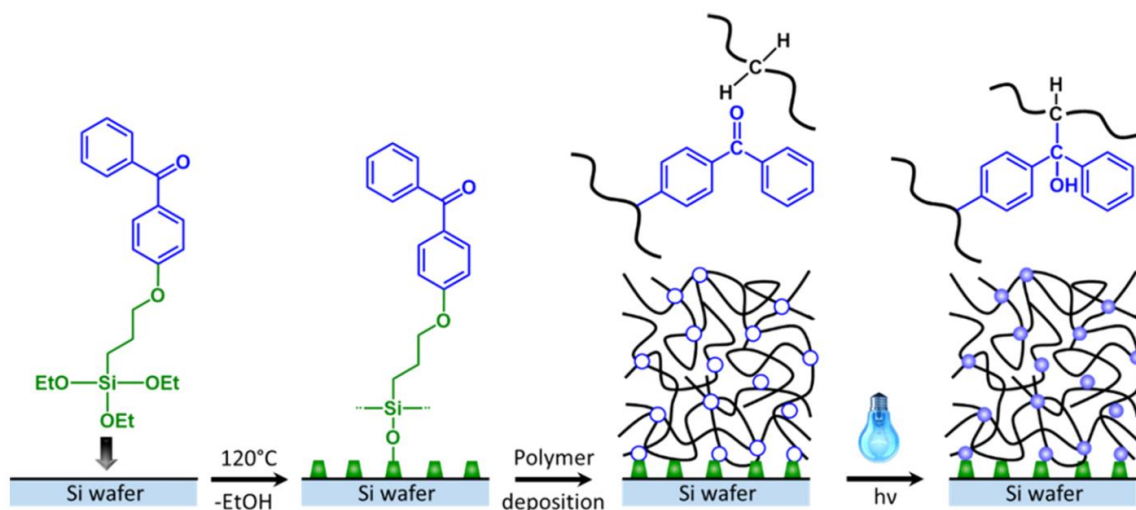
harder to control the thickness of the brushes. On the other hand, the films formed by spin coating can be controlled and reproduced much easier. For instance, the thickness of films can be controlled by changing the spinning rate. Thicker films are formed using lower spin rates, and thinner films are formed by using faster spin rates. Additionally, one can control the thickness of polymer layer by changing the solvent and the concentration of the solution. Additionally, spin coating usually takes around a minute to form a uniform film. This behavior contrasts with brush formation, which takes hours or longer. After it was determined that spin coating would be a superior method of depositing a thin film, the next challenge was to find a method to anchor the polymer to the surface.

3.1.2 Benzophenone as an anchor

One possibility of anchoring the polymer to the surface was to use UV light in order to generate radicals that would react with the polymer. It was determined that benzophenone (BP) would be an ideal candidate for this because of its stability and widely studied ability to react with C-H bonds.^{1,2} Additionally, BP modified with silanes has been reported so there is a known methodology to covalently bound it to silicon substrates.³ We envisioned that once the BP-silane has been deposited on the surface, a polymer can be spun cast on top of the silane monolayer and then crosslinked, thus covalently binding the polymer to the surface. However, it should be noted that this will only react the polymer that is in contact with the BP-silane. The rest of the polymer will wash off the wafer. Thus, in order to link the entire polymer film to the surface, we envisioned that BP must be incorporated into the polymer chain as well. Thus, after UV treatment, the polymer is

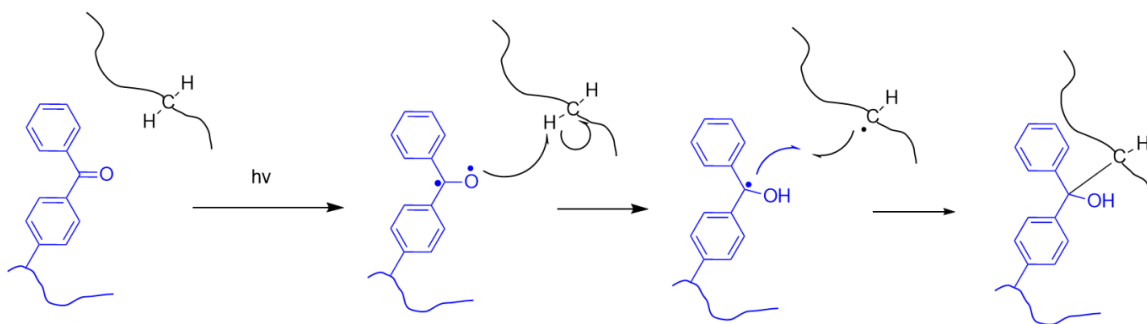
anticipated to crosslink with itself and attach to the surface. This process is shown below in Scheme 3.2.

Scheme 3.2. BP-Silane Deposition and Polymer Deposition with UV-Crosslinking⁴



When benzophenone reacts with UV light, it generates a di-radical like excited state across the carbonyl bond. This di-radical can then abstract a hydrogen from the polymer chain and will react with the carbon in the polymer chain that lost the hydrogen. It is important to note that one benzophenone can only do this process once and then terminates, it is not a radical chain process that is often observed with radical reactions. A mechanism of the process is shown below in Scheme 3.3.

Scheme 3.3. BP-Silane Photocrosslinking mechanism



3.1.3 Goals

The goal of this work is to create a spun cast and crosslinked polymer network. Once the network is established, it will be subjected to degradation studies by aqueous solutions at different pH values. The ability of the modified surface to resist protein adsorption will be tested as well. The ultimate goal is to create a polymer network that both degrades slowly over time at a biological pH of 7.4 and can also resist protein adsorption.

3.2 Results and Discussion

3.2.1 Initial Attempts at forming Spun Cast Thin Films

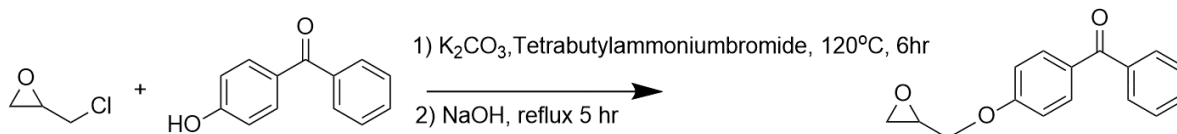
It was decided to continue with a similar type of anhydride/epoxide alternating copolymer that was employed in Chapter 2. Poly(maleic anhydride-co-ETEG) was synthesized according to the procedures listed in Chapter 2, yielding polymer with a molecular weight around 4,000 Da. The first attempt at creating films was to create blends of the polymer and BP. This blend was then spun cast onto BP-silane wafers. The blend of polymer and BP was subjected to solubility tests in order to find a suitable solvent for spin

coating. THF was determined to dissolve both the polymer and the BP. However, when spin coating the blend, the polymer phase separated with the BP and produced very poor films. Additionally, THF is a poor solvent to use for spin coating because it has a low boiling point. This allows for the solvent to evaporate too quickly and destroys the uniform quality of the film. As a result of this, dip coating the blend was attempted on BP-silane wafers. This process again produced phase separated films, but these were nonetheless subjected to UV-photocrosslinking. The crosslinked wafer was washed with THF twice and the polymer remained visible on the substrate. When uncrosslinked wafers were washed with THF, the polymer was visibly removed from the substrate. Although the quality of the films was poor, this result indicated that the BP-silane wafers were capable of reacting with the polymer and that the BP was capable of forming crosslinked networks with this polymer. However, in order to improve the quality of the films, it was determined that BP needed to be incorporated into the polymer chain.

3.2.2 Synthesis of Epoxy-Benzophenone

To incorporate a BP moiety into the polymer backbone requires synthesis of a BP-containing epoxide or anhydride. As study of other anhydrides such as succinic anhydride or diglycolic anhydride were planned, synthesis of a BP-epoxide was the more attractive option. As it turned out, reacting epichlorohydrin with 4-hydroxy benzophenone has been reported multiple times in the literature.^{5,6} Two synthetic routes were attempted shown below in Scheme 3.4.

Scheme 3.4. Synthesis of epoxy-benzophenone (EBP)



The first attempt gave the pure product, but produced small yields of 8-20%. After this, the second approach gave a yield of 87%. Additionally, the 1H NMR data matched what was reported in the literature very closely and the results are listed below in Figure 3.1.

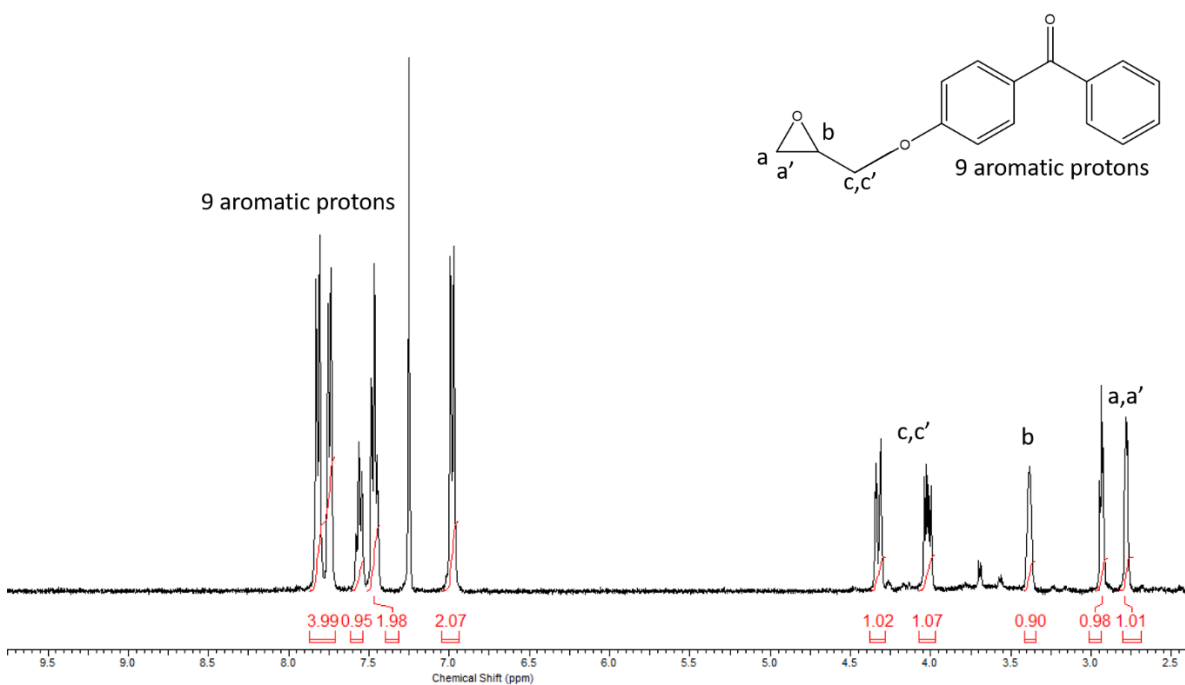
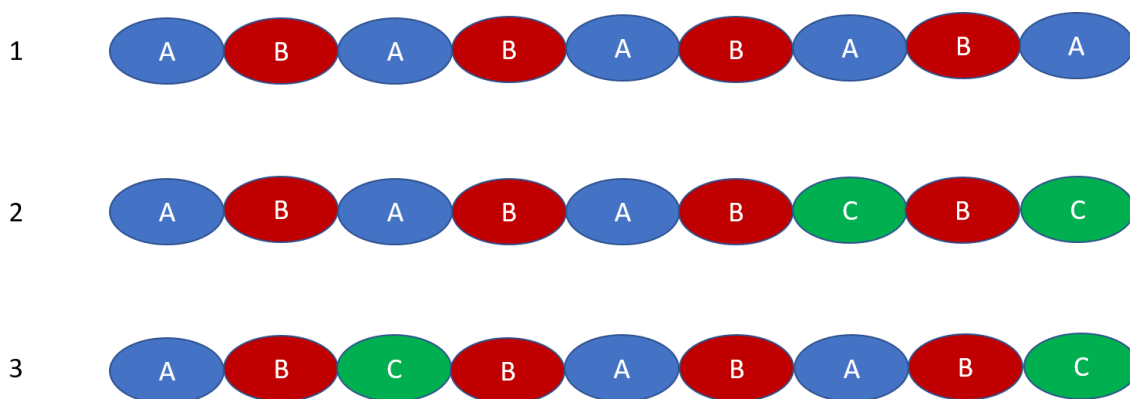


Figure 3.1. 400 MHz 1H NMR of Epoxy-benzophenone in $CDCl_3$

3.2.3 Reaction Rates of Terpolymerizations

With the synthesis of EBP completed, it was determined that a small weight percentage of EBP was desirable for incorporation into the poly(maleic anhydride-co-EPEG) polymer. However, one potential problem arises as a result of a terpolymerization in which there are three different co-monomers. If the catalyst has a preference for one epoxide over another, then control over the percentage of incorporation can be difficult. An example of this is shown below in Scheme 3.5.

Scheme 3.5. Different Types of Copolymerizations



For example, copolymer 1 represents a typical copolymerization in which there is one epoxide (A) and one anhydride (B). In example 2, there are two epoxides (A and C) but the catalyst has a preference for A. Once A is used up, the catalyst starts to incorporate C. This results in a gradient copolymer where there are distinct regimes of each epoxide. Copolymer 3 shows a truly random copolymer where the catalyst has no preference for A over C or vice versa. A random copolymer is the most desired copolymer in this application because the EBP will be incorporated evenly across the polymer and thus give a better polymer network.

In order to test the affinity of one epoxide over another, a terpolymerization of maleic anhydride, ETEG and allyl glycidyl ether (AGE) was conducted. AGE was chosen because of the diagnostic protons on the allyl group. By integrating the protons on the allyl group and comparing them to the integration of the protons of the methyl on the ETEG, the amounts of each can be calculated and compared. The results of that experiment are listed below in Table 3.1. The data indicates that there is a slight preference for ETEG over AGE but not a large preference. These results are indicative of a random copolymer and were desirable enough to continue with incorporating EBP into the polymer chain.

Table 3.1. ETEG vs AGE incorporation into terpolymer

| Time (h) | ETEG:AGE ^a |
|----------|-----------------------|
| 2 | 1.2:1 |
| 4 | 1.3:1 |
| 6 | 1.2:1 |
| 24 | 1.1:1 |

MA:ETEG:AGE:Cat 200:100:100:1, 45 °C, ^a Calculated by integrating protons on each and dividing them by the number of theoretical protons.

3.2.4 Incorporation of EBP into Polymer

Incorporating EBP into the polymer chain is a key factor that should significantly improve the quality of the spun cast films. During the first attempt, 25% EBP was added to polymerization reaction. The ¹H NMR of the resulting polymer is pictured below in Figure 3.2.

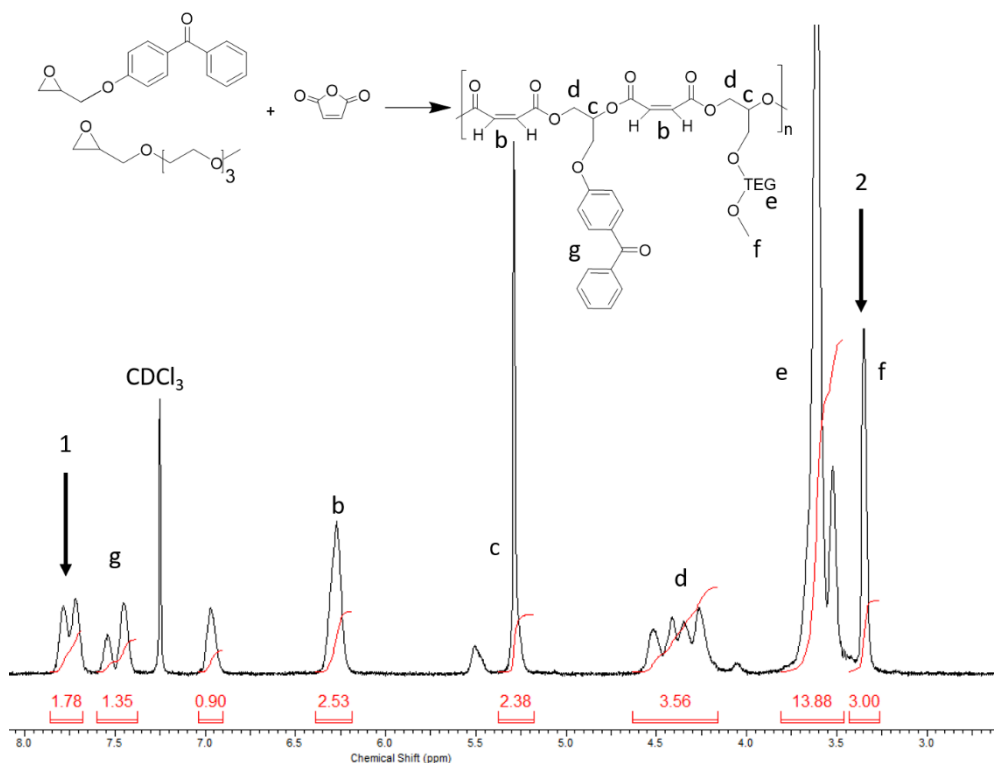


Figure 3.2. 400 MHz ^1H NMR of 25% EBP feed Ratio in Poly(MA-EETEG) in CDCl_3

Peaks labeled 1 and 2 are the diagnostic peaks of the epoxides. Peak 1 is the aromatic protons on EBP. Peak 2 is the methyl group on the ETEG. When comparing the integrations with the calculated value and then dividing by the sum, the polymer pictured above shows that it is made up of 30%MA, 48% ETEG and 22% EBP. The feed ratio of EBP was 25% so there was a slight loss of incorporation but nevertheless an acceptable result. This methodology represents how the different percent incorporation of EBP was determined for all the other feed ratios. The 25% feed was chosen to show because it is the easiest to visually explain the process.

For all the terpolymers, the percent incorporation of EBP compared to the feed ratio was always less, but only by a few percentage points. Additionally, all of the polymers

exhibited a higher percentage of ETEG than calculated and a lower percent incorporation of MA. This might have to do with the catalyst having a slight preference of ETEG over the other monomers as shown above. Listed below in Table 3.2 is the data for all the different feed ratios of EBP.

Table 3.2. Summary of EBP feed ratios, incorporation and M_w

| EBP Feed Ratio (%) | EBP Incorporation (%) ^a | M_n (Da) ^b | M_w (Da) ^b | PDI ^b |
|--------------------|------------------------------------|-------------------------|-------------------------|------------------|
| 25 | 22 | 3000 | 3800 | 1.3 |
| 10 | 8 | 2200 | 2900 | 1.3 |
| 2.5 | 1.7 | 2200 | 2700 | 1.2 |
| 1 | 0.5 | 2000 | 2500 | 1.3 |

^a Determined by ¹H NMR, ^b Determined by GPC

3.2.5 Gel Fraction

After the films were allowed to anneal overnight, the gel fraction was determined. This value is the thickness of the washed wafer divided by the thickness of the unwashed reference wafer. In order to determine the gel fraction, the wafers were washed with copious amounts of dioxane and then dried with a stream of nitrogen gas. Below in Table 3.3 is the gel fractions for all of the different feed ratios of EBP.

Table 3.3. Gel Fraction of different EBP feed ratios

| EBP Feed Ratio (%) | Gel Fraction (%) ^{a,b} |
|--------------------|---------------------------------|
| 25 | >95 |
| 10 | >95 |
| 2.5 | >93 |
| 1 | 60 |

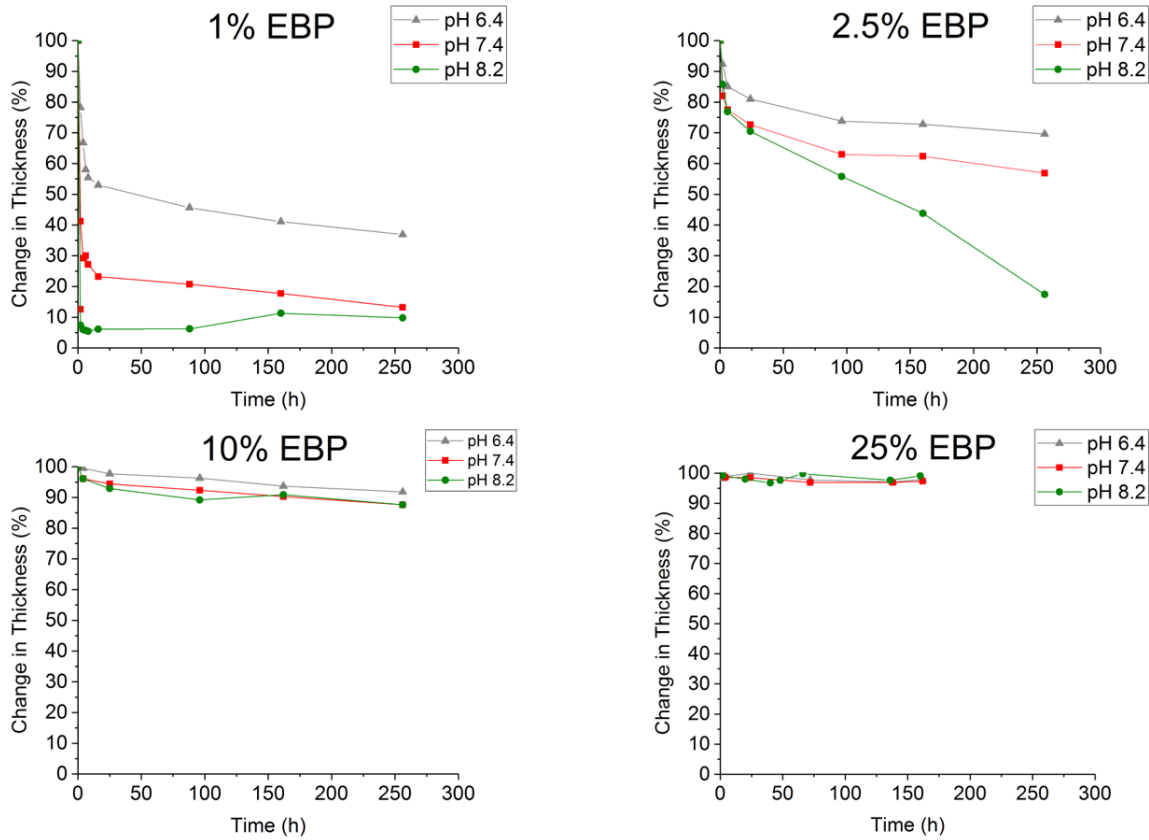
^a Determined by ellipsometry. ^b Gel Fraction= (washed thickness)/(unwashed thickness).

The 10% and 25% EBP feed ratios indicate that the films are extremely crosslinked as very little of the film washed away. The 2.5% EBP feed ratio indicates that the film was almost as crosslinked as the higher feed ratios but enough EBP was added to maintain the majority integrity of the film. However, the 1% EBP feed ration indicates that the film was not sufficiently crosslinked as about 40% of the thickness was washed away. After the gel fractions were calculated, the wafers were subjected to degradation studies.

3.2.6 Degradation and Protein Studies

For the degradation studies, the one wafer was placed in a vial with 10 mL of a phosphate buffered saline (PBS) solution at a pH of 6.4, 7.4, and 8.2 of an ionic strength of 0.015 M. Additionally, one wafer was placed in a vial with 10 mL of a bovine serum albumin (BSA) in PBS solution at a pH of 7.4. The vials were then placed on a shaker table 200 rpm for the indicated amount of time. Periodically, the wafers were removed, washed with copious amounts of water and blown dry. The thicknesses were then determined by ellipsometry.

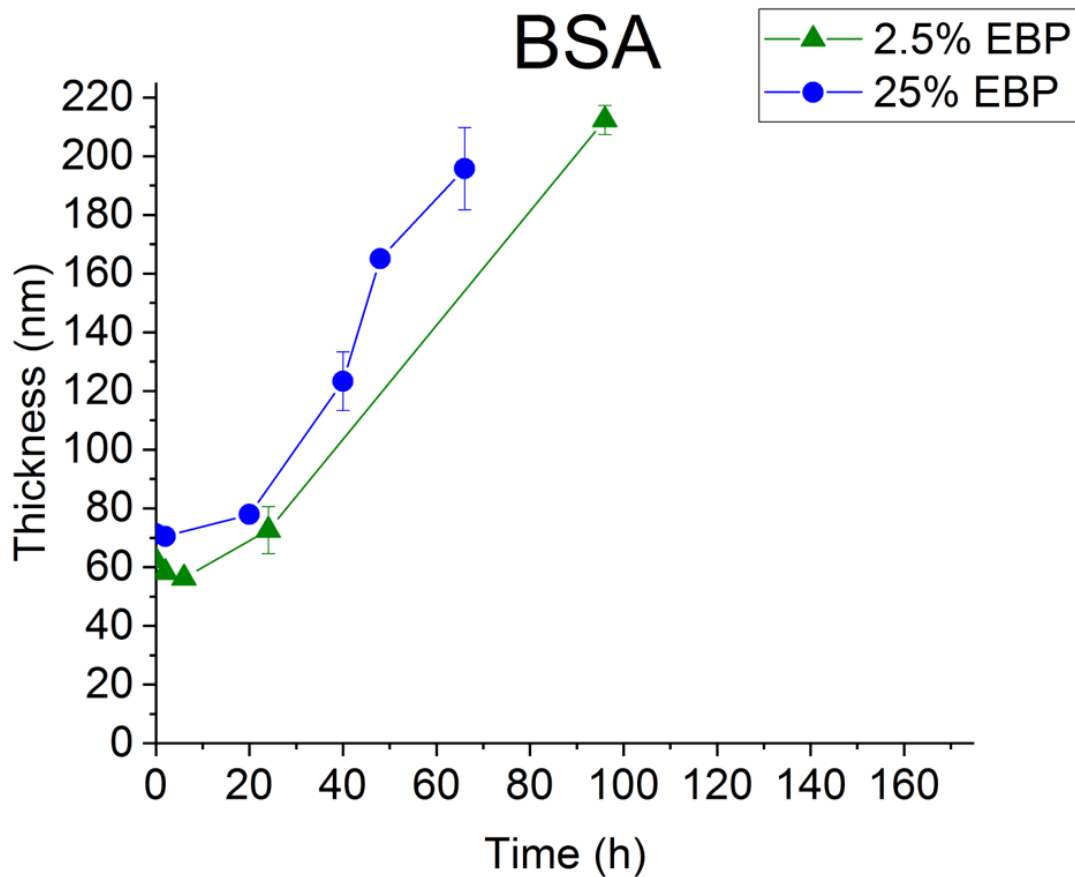
Scheme 3.6. Thickness vs. Time for polymer layers prepared with different feed ratios of EBP. Lines are added to help guide the eye and do not represent a fit to the data.



As indicated above in Scheme 3.6, there is a clear trend of degradation for the different pH values and EBP feed ratios. The layer on the wafer exposed to pH 6.4 buffer degraded the slowest, followed by that exposed to pH 7.4 buffer. The layer exposed to pH 8.2 buffer degraded the fastest. When comparing the different feed ratios, layers composed of the 10% and 25% EBP were very similar in that very little of the layer degraded over time. This behavior suggests that these layers were too crosslinked to allow for degradation over the time scales investigated. Conversely, the layer composed of 1% EBP showed rapid degradation after less than 24 hours. This behavior is likely due to insufficient crosslinking and the film was destroyed. Finally, the layer composed of 2.5% EBP showed a degree of a

controlled degradation over time, which is an ideal behavior. Scheme 3.7 shows a low EBP feed ratio of 2.5% and a high EBP feed ratio of 25% and the ellipsometric thickness over time. In both cases, protein adsorption occurred very rapidly and resulted in an immeasurable thickness after only 100 hours.

Scheme 3.7. Thickness vs. Time of wafers when exposed to protein solution. Lines are added to help guide the eye and do not represent a fit



3.3 Conclusions

3.3.1 EBP Blends

Creating blends of benzophenone and the poly(MA-co-ETEG) proved to be an undesirable method when spin coating onto BP-silane wafers. The polymer and BP phase separated, giving poor quality films.

3.3.2 Synthesis of EBP

Synthesizing EBP proved to be relatively straight forward. Although the first method did not work, the second method proved to be much simpler and gave much better yields.

3.3.3 Reaction Rates and EBP Incorporation

The reaction rate experiments determined that the catalyst has a slight preference of incorporation ETEG over AGE. Additionally, ETEG was incorporated more over MA in the case of both sets of terpolymerizations. It is unclear why this is the case and might be an interesting to further investigate in the future.

3.3.4 Gel Fraction

The gel fraction experiments indicated that the EBP was an effective crosslinking agent used under the conditions described more fully in the experimental section. Additionally, it also proved that BP-silane is an effective surface anchor for this system. If either the EBP or the BP-silane proved to be ineffective, then the dioxane wash would have removed the vast majority of the films.

3.3.5 Degradation Studies

The degradation studies showed that an ideal EBP feed ratio of those investigated was 2.5% EBP during the time scales of the experiments. The 10% and 25% EBP feed ratio gave films that were too crosslinked and did not degrade over an appreciable amount of time. If the films do not degrade, then they are not able to resist protein adsorption over time in a controlled manner. The 1% EBP feed ratio gave a film that was too little crosslinked, and degraded very quickly. All of the films showed less degradation at pH 6.4, moderate at pH 7.4 and the fastest rate at pH 8.2. This data matches what has been reported on previously with polyesters degrading fastest under basic conditions.⁷ The 2.5% EBP feed ratio showed the best results of a controlled degradation over time.

3.3.6 Protein Adsorption Studies

Unfortunately, all of the wafers failed to resist protein adsorption. The wafers started to show significant protein adsorption after just 10 hours. Additionally, it was hard to determine the thickness increase over longer time scales because the thickness began to prevent an accurate measurement by the ellipsometer. One potential cause of the protein adsorption may be to the use of maleic anhydride as a co-monomer. Maleic anhydride contains a double bond that is a hydrophobic moiety and when opened, would allow for the creation of a hydrophobic backbone along the polymer chain. Proteins have been shown to adhere to hydrophobic surfaces with relative ease. Switching out the maleic anhydride with a hydrophilic monomer might change the behavior of the films. A hydrophilic polymer backbone would allow for the formation of a better hydration sphere that would create

more resistance to proteins. Additionally, the film itself might swell more off of the surface, allowing more water molecules to penetrate the film network. This approach would also have the added benefit creating more obstacles for the proteins. However, switching monomers would present a series of new challenges that need to be explored. To start, changing the monomer might change the polymerization and the catalyst might behave differently. Additionally, the spin coating might be different as BP-Silane is hydrophobic, which might induce phase separation. The crosslinking might also be different as the double bond in MA likely provided a great site for radicals to react with. Finally, the degradation kinetics might change as well. All of these potential issues would need to be addressed in the future.

3.4 Experimental

3.4.1 Solvents and Glassware

All solvents were used as received. All glassware was washed with acetone, soap and water and then acetone again. All glassware was either flame dried or oven dried and moved into the glovebox while still hot.

3.4.2 Instrumentation

Polymers were characterized by NMR with a 400 MHz Varian Inova 400 spectrometer at room temperature. Polymer molecular weights were obtained with a PL-GPC 50 Plus using a Jordi-Gel DVB 1000A column with a refractive index detector. The solvent used was distilled THF at 1 mL/min at 35°C. Near monodisperse polystyrene standards were obtained from Fluka ranging from 250-70000 Da. Spin coating was done

using a PNM32 model Headway Research spin coater. Crosslinking was accomplished with an Omnicure series-1000 UV Lamp at a wavelength of 365 nm. from Lumen Dynamics. The UV light intensity was measured with a ILT1400-A photometer. Wafer thickness was determined by use of Variable-Angle Spectroscopic Ellipsometry (VASE, J.A. Woollam, USA). The model used to fit was a simple Cauchy layer. Angle of measurement was 70°.

3.4.3 Solution Terpolymerization

A typical terpolymerization was as follows: inside a dinitrogen filled glovebox, 0.02 mmol of (R,R)- N,N'-Bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexanediaminochromium(III) chloride (Cr(III) (salen)), 4.0 mmol of sublimed maleic anhydride, 4.0-X mmol of ETEG, X mmol of EBP, ~ 1 mL of hexanes and a stir bar were added to a 20 mL scintillation vial. A screw cap with a teflon liner was then added to the vial, and the vial was sealed with electrical tape. The solids in the vial were then allowed to dissolve and then the vial was placed in an oil bath at 45°C overnight while stirring using a magnetic stir bar. The vial was opened and a minimal amount of DCM was added until all the polymer was dissolved. The polymer was then precipitated in about 50 mL of diethyl ether. The liquid was decanted off into centrifuge tubes and spun at 2500 rpm for 10 minutes. The liquid was poured off and the polymer at the bottom of the centrifuge tubes was combined. The solvent was removed with rotary evaporation followed by exposure to high vacuum. The polymer was characterized with a 400 MHz ¹H NMR and by gel permeation chromatography using a refractive index detector in THF.

3.4.4 Wafer Preparation

Mechanical grade silicon wafers were obtained from Silicon Valley Microelectronics with an orientation of 100°, thickness of 0.5 mm, and diameter of 100 mm and were cut into 1.5 to 2.0 cm squares. The wafers were then cleaned and hydroxylated with UV-Ozonolysis treatment for 30 minutes. The BP-silane was obtained from Dr. Genzer's group and used as received. BP-silane (30 mmol) was freshly prepared in toluene and spun-cast onto the UVO-cleaned silicon substrate. The sample was then annealed at 120 °C for 3 h, followed by extraction in toluene and drying under N₂ gas. This procedure yielded a monolayer of BP-silane (thickness 2–3 nm) on the silicon wafer.⁴

3.4.5 Spin Coating

Polymer was dissolved in dioxane at a concentration of 20 mg/mL. The solution was then run through a 0.2 µm filter. Then, a BP-silane wafer was placed on the spin coater and spun at 2500 rpm for 30 seconds at a 1500 ramp up spinning rate. While spinning, ~1 mL of dioxane was dropped on the wafer in order to clean it. Once stopped, the polymer solution was placed on the wafer, fully covering it and spun under the same conditions. After the wafers were coated, they were covered loosely with tin foil and allowed to anneal at room temperature overnight. The following morning, the wafers were washed with copious amounts of dioxane. One wafer was kept unwashed to use as a reference for the gel fraction. The thickness of the layers on the wafer were determined by ellipsometry.

3.4.6 Buffer Preparation

Buffer solutions were prepared by dissolving 0.6 g of NaH_2PO_4 in 450 mL of DI water. Then 4.052 g of NaCl was added to give the solution a 0.015 M ionic strength. NaOH was added to bring the pH to the desired amount and 50 mL of water was added to bring about a 0.01 M solution of PBS buffers at pH 6.4, 7.4 and 8.2.⁸ For the bovine serum albumin (BSA) solution, 260 mg of NaH_2PO_4 and 451 mg of monobasic sodium phosphate heptahydrate to 180 mL of DI water. Then, 506 mg of NaCl was added and the pH adjusted to 7.4 by adding NaOH. The total volume was brought to 200 mL with DI water. In a volumetric flask, 451 mg of BSA was added and dissolved in 100 mL of the solution.⁹ The flask was then stored in the refrigerator until use.

3.4.7 Degradation Studies

Wafers with thin films were immersed in a vial with 10 mL of the 4 different solutions. These were placed on a shaker table at 200 rpm at room temperature. At the indicated time interval, the wafers were removed from the vials, washed with copious amounts of DI water and blown dry under a stream of nitrogen gas. The thickness was determined with ellipsometry. Two ellipsometry measurements were taken and if the thickness difference was +/- 1 nm then the first measurement was used. If the thickness of the two measurements was larger than a 1 nm difference, then one more measurement was taken. The thickness reported is an average of the 3 measurements and error bars indicate this range.

3.5 References

- (1) Christensen, S. K.; Chiappelli, M. C.; Hayward, R. C. Gelation of Copolymers with Pendant Benzophenone Photo-Cross-Linkers. *Macromolecules*. **2012**, *45* (12), 5237–5246.
- (2) Pandiyarajan, C. K.; Prucker, O.; Rgen, J.; He, R. Humidity Driven Swelling of the Surface-Attached Poly(N-alkylacrylamide) Hydrogels. *Macromolecules*. **2016**, *49*, 8254-8264.
- (3) Prucker, O.; Naumann C. A.; Rhe J.; Knoll, W.; Frank, C.W.; Photochemical Attachment of Polymer Films to Solid Surfaces via Monolayers of Benzophenone Derivatives. *J. Am. Chem. Soc.* **1999**, *121*, 8766-8770.
- (4) Pandiyarajan, C. K.; Genzer, J. Effect of Network Density in Surface-Anchored Poly(N-isopropylacrylamide) Hydrogels on Adsorption of Fibrinogen. *Langmuir*. **2017**, *33*, 1974-1983.
- (5) Yang, J.; Tang, R.; Shi, S.; Nie, J. Photochemical & Photobiological Sciences Synthesis and Characterization of Polymerizable One-Component Photoinitiator Based on Sesamol. *Photochem. Photobiol. Sci* **2013**, *12*, 923-929.
- (6) Jabeen, I.; Pleban, K.; Rinner, U.; Chiba, P.; Ecker, G. F. Structure–Activity Relationships, Ligand Efficiency, and Lipophilic Efficiency Profiles of Benzophenone-Type Inhibitors of the Multidrug Transporter P-Glycoprotein. *J. Med. Chem.* **2012**, *55*, 3261-3273.

- (7) Hu, X. Investigation of Growth and Degradation Behaviors of Biodegradable Polyester Brushes and Their Applications. Ph.D. Dissertation, North Carolina State Univ., **2013**.
- (8) <http://www.biomol.net/en/tools/buffercalculator.htm>
- (9) Daye, A.J.; Synthesis of Protein-Resistant, Biodegradable Polymer Brushes, M.S. Dissertation, North Carolina State Univ., **2016**.