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Immobilization of Biomolecules on Polymer Scaffolds: A Novel Approach

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IMMOBILIZATION OF BIOMOLECULES ON POLYMER SCAFFOLDS: A NOVEL APPROACH

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ABSTRACT

In this thesis, I describe the successful development of a procedure for the step-by-step formation of a multi-layer polymer scaffold on a silicon wafer and the characterization of these materials. Also discussed is the development of a procedure for the non-site specific attachment of a biomolecule to the modified silicon wafer, including scaffolds modified via drop-on-demand, DOD, inkjet printing. Ellipsometry, x-ray photoelectron spectroscopy (XPS), FTIR, fluorometry, and static water contact angle measurements are used to study the nanoscale structure and properties of the interfacial, thin film-modified surfaces. Polymers based on 2-vinyl-4,4-dimethylazlactone (VDMA) are used as the platform onto which biomolecules are tethered. This monomer is a novel material for bioscaffolds, and advantageous because of its high conversion from monomers, ability to copolymerize, and unlike polymers bearing *N*-hydroxy succinimide esters, poly(2-vinyl-4,4-dimethylazlactone) (PVDMA) is hydrolytically stable. Since vinylpyrollidone (VP) has low toxicity, biocompatibility, and the ability to improve the solubility of the polymers in water, it is copolymerized with VDMA. The base of the multilayer polymer scaffold is poly(glycidyl methacrylate) (PGMA), which has demonstrated outstanding success in attachment to silicon wafers and subsequent modification by small molecules and polymers. Dansylcadaverine is used as a model biomolecule for attachment because it has a single primary amine, and is fluorescent, which allows for easy characterization. The spectroscopic characterization in conjunction with the ellipsometric and static water contact angle results confirm the anticipated

structure. Additionally, fluorometry shows the successful biomolecule attachment onto the multi-layer scaffold. The protocol presented here is applicable for attaching a variety of amine-containing biomolecule to the modified-surface for a wide array of applications.

DEDICATION

This thesis is dedicated to my parents, Michael and Susan, my sister, Brooke, my friends, and all those who supported and encouraged me along the way.

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Thank you to my advisor, Dr. S. Michael Kilbey, II, and my committee members, Dr. Douglas Hirt and Dr. Igor Luzinov for the time and effort spent on this thesis and defense. Much gratitude is given to Dr. Jamie Messman for synthesizing the copolymers and characterizing the samples via ATR-FTIR and XPS. Special thanks are also extended to Mary Alice Salazar for obtaining fluorometry results, and Dr. Amit Sankhe for instructing me in the use of the DOD inkjet printer.

I could not have achieved this goal without the support, love, and encouragement of my family and friends. Thank you to my family for teaching me that with faith, hard work, and persistence, you can reach your goals. I also greatly appreciate the help and encouragement of my friends Jason and Tarra, Patrick and Christine, Ronnie, Jeff, Brooke, and Nichole. The research group, Santosh, Jose, Juan Pablo, Azedeh, and Alaina have been a joy to work and converse with. I also thank the many other friends, professors, and staff that have helped me during graduate school. Most of all I thank my best friend and Savior, Jesus Christ, through whom I have gained strength to do all things and to whom I give all the glory of completing this work.

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CHAPTER 1

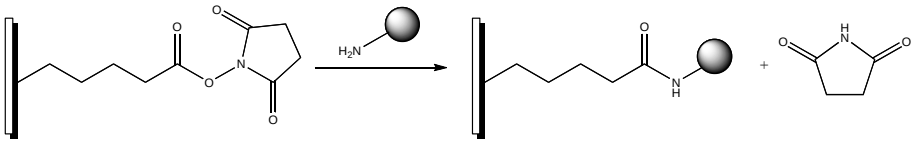
INTRODUCTION

Background

Polymeric materials are used in a variety of biomedical applications like biosensors, restoratives for the repair of teeth and bones, bio-regulation, and drug delivery.[1-4] More specifically, surfaces are frequently modified with biomaterial coatings to enhance therapeutic functionality, improve biocompatibility, and locally deliver treatment via implantation.[3] My thesis work investigates a method to immobilize biomolecules onto a solid support created by a multi-layer polymer scaffold. This approach employs a nonspecific strategy to attach biomolecules to a modified surface. In site-specific reactions, one needs to design a polymer with reactive groups to target one specific site on the biomolecule. However, there are many sites on biomolecules that can be utilized as anchoring points for immobilization. Therefore, precise and laborious organic and biochemical syntheses are required. Nonspecific strategies offer the ability to attach a biomolecule without having to create a different surface layer to target specific biomolecules. This means that the surface can be utilized for the attachment of a range of biomolecules and can be used in a variety of applications. For this reason, nonspecific attachment of biomolecules to polymer-modified surfaces is widely practiced.

One such strategy used for the nonspecific immobilization of biomolecules is *N*-hydroxy succinimide (NHS) ester technology, which has proven to be a very successful method.[4] In this approach, activated esters are used for the covalent coupling of

biomolecules. Scheme 1.1 illustrates the coupling of amine groups with NHS esters. This strategy has been implemented with self-assembled monolayers (SAMs) on surfaces, as well as free and surface-tethered polymers.[4] The difficulty with this method is that after a monomer has been selected for surface modification, a polymerization method must be chosen that will not cause the NHS esters to react. In order for a biomolecule to attach to an NHS-modified surface, the biomolecule must be dissolved in a low-ionic-strength buffer. Then an amine group of the biomolecule can react with the NHS esters, forming stable amide bonds and liberating the succinimide byproduct. The efficiency of the reaction depends upon pH, concentration, ionic strength, and reaction time. For each type of protein studied, these parameters need to be explored to find the most favorable reaction conditions.[5]



Scheme 1.1. Amine chemistry of NHS ester modified surface.

However, there are issues with this approach.[6] It was noticed that NHS esters compete for available attachment sites for the amine coupling reaction needed for biomolecule attachment. In order to prevent NHS esters from such competition, they need to undergo hydrolysis, which renders the NHS ester inactive. The major hindrance is that in order to conduct this hydrolysis, the conditions required are so aggressive, the effects are felt throughout the rest of the material to which it is attached. Also, Wong and

Putnam[7] recently described in detail how functionalization of NHS-bearing polymer is complicated by side reactions, including ring opening of the succinimide group and glutarimide formation between neighboring sites. Additionally, polymers containing NHS esters have limited water solubility and they are not stable.[7,8] While these problems do not preclude the use of NHS esters for developing bioconjugates, it could be argued that other approaches that are more robust are worthwhile.

Research Objectives

The main goal of this research is to develop a novel approach for the nonspecific biomolecule immobilization on polymer scaffolds and characterize the structure and properties of the scaffold. In this research, I have developed a method for coating surfaces with random copolymers capable of being functionalized with biomolecules and explored the non-selective attachment of a representative biomolecule on this interfacial layer. This surface-modifying layer provides the capability to attach a biomolecule through an easy nucleophilic addition reaction that results in the formation of a strong covalent bond between the biomolecule and the scaffold. The specific objectives of this work include: development of a step-by-step procedure for multi-layer polymer scaffold formation; characterization of each step in the scaffold preparation procedure; development of attachment procedure of a biomolecule to the scaffold-modified silicon wafer; and characterization of the modified wafer after biomolecules have been attached, by methods including drop-on-demand, DOD, inkjet printing. The process is done on surfaces rather than in solution because of the many characterization methods available

for studying surfaces. I am able to utilize x-ray photoelectron spectroscopy (XPS), FTIR, ellipsometry, and static water contact angle measurements to study the molecular structure and properties of the surfaces.

In this work, polymers based on 2-vinyl-4,4-dimethylazlactone (VDMA) are used as a platform onto which biomolecules can be tethered. One of the most useful properties of VDMA is its high conversion of monomers to polymers without the need for chain transfer agents for effective management of molecular weight.[9] The pendant azlactone rings react to form stable covalent bonds with amine and thiol groups commonly found in enzymes, proteins, and other biomolecules.[10-11] As previously mentioned, one of the drawbacks of NHS esters is its limitations in water solubility and stability. However, in my research, it was noticed that some of the VDMA-based copolymers are water soluble (3:1) and hydrolytically stable, keeping the functionality of the azlactone rings. Nucleophilic addition of a primary amine proceeds without a catalyst and at room temperature, yielding a stable amide linkage.[4,11] This property has been exploited to immobilize proteins VDMA polymers in solution.[11] Stanek et al.[12] successfully created a copolymer system with N,N-dimethylacrylamide (DMA) and VDMA. VDMA monomer can be polymerized without the concern of destroying its functionality, unlike the aforementioned attribute of NHS esters, where choice of monomer and polymerization is an issue. Because of this attribute, VDMA can be incorporated into variety of polymer architectures, like block copolymers, which is not possible with NHS esters, as no reports of such a polymer can be found.

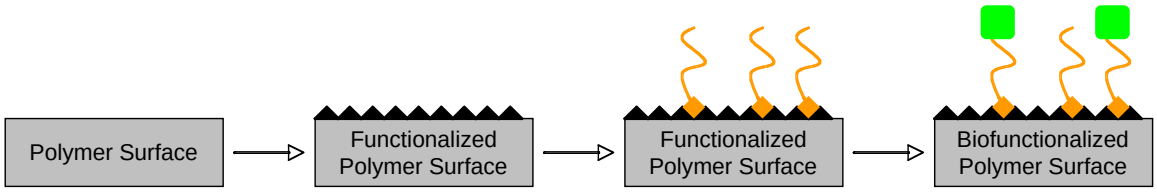
In this research, VDMA was copolymerized with vinylpyrollidone (VP) because of its low toxicity, biocompatibility, and ability to improve the solubility of the polymers in water.[13] Poly(glycidyl methacrylate) (PGMA) was used as the base of the polymer support because of noted success in attachment to silicon wafers.[14] PGMA is also known for its high surface density and affinity for reacting with amines.[15] Dansylcadaverine was chosen as a model molecule for studies of biomolecule attachment because it has a single primary amine and is fluorescent,[16] thereby simplifying attachment and facilitating characterization.

Our research group has demonstrated success with drop-on-demand (DOD) inkjet printing to create patterned, interfacial polymer layers.[17] There is great potential for advancements in biomaterials created via this method. Thus, the attachment of a biomolecule to the multi-layer polymer scaffold was explored.

Surface-Tethered Polymers for Biomedical Applications

A considerable amount of effort has been directed at creating bio-responsive or biocompatible surfaces by the covalent immobilization of bioactive compounds onto functionalized polymers. The most common scheme for building a polymer-modified surface that is functionalized with a biomolecule is shown in Scheme 1.2. In this scheme, the general idea is to choose a polymer with the appropriate properties for meeting the specific needs of the end use. Oftentimes, the polymers must first be functionalized so that the target biomolecule may be subsequently attached to the surface. This step allows

for the specialization of the overall modified surface, such that the degree of functionalization of the polymer layer or surface density of biomolecules can be tailored.



Scheme 1.2. General idea for surface modification with non-specific biomolecule attachment.

The advantageous aspect of covalent immobilization is the ability to provide a stable bond between the biomolecule and the functionalized polymer. Covalent bonding ensures integrity of the layer, promoting prolonged and/or continual activity of implanted devices.[6] A particular challenge is ensuring that the chemical functionalities necessary for a subsequent step are not consumed or degraded in any prior step or upon exposure to the various solution environments used.

Modification of Surface Chemistry

Ionized gas treatments consist of plasma, corona discharge, flame treatment, and UV irradiation. Plasma treatment has the ability to modify the top nanometer of a surface without the use of solvents. However, plasma activation requires the use of a vacuum to remove any latent gases and low repeatability is also a key hindrance in using plasma treatments. In the corona discharge technique, a stream of ionized particles is introduced

to the polymer surface. This method, however, does allow for possible contamination because it is conducted under ambient conditions and temperature, and humidity can greatly affect the outcomes of the treatment.[6]

In flame treatment, reactive oxygen is created by burning a gas that is rich in oxygen. Even though flame treatment is promising because it is cheap, many factors must be controlled with fine precision in order to obtain consistent results.[6] UV irradiation relies on the polymer surfaces generating site that, when exposed to gas, become functional groups. This method requires the depth of surface reactivity to be controlled. Oftentimes, particles can prohibit UV light reaching the surface, thereby affecting the repeatability of the treatment and a variety of reactions occur upon irradiation, damaging the polymer and leading to the creation of different surface chemistries.

In addition to these methods, additive migration is also a viable option for modifying a surface. Additive migration involves incorporating a second species, different both chemically and physically, into a polymer matrix, and then allowing that second component to migrate to the film surface.[18] Minimizing the total free energy of the system drives additive migration. The total energy is comprised of the entropy of mixing, the interaction energy between the system components, and the surface energy.[19] The thermodynamics of the system can be changed by altering the molecular weight and chemical functionality of the additive, molecular weight and crystallinity of the polymer matrix, as well as the surroundings of the system.[20-21] A challenge with this approach is ensuring the correct formulations and conditions to achieve the proper orientation and surface density of the desired species at the surface.

Modification of Surfaces Using Polymers

The wet chemical approach entails treating the surface with liquid reagents to impart reactive functional groups on the surface. Even though this technique does not require specialized equipment, a major drawback is that it is non-specific and often can generate a range of functional groups. Another downside to the wet chemical method is the low repeatability when characteristics of the polymer change. Silane or thiol monolayers have the quality of being able to self-organize at hard, inorganic surfaces, forming a single layer. Surface properties can be changed by using a monolayer-forming species that contains a functional group at the terminus that is complementary to (does not react with) the anchoring group that covalently bonds the molecules to the surface. The structure of SAMs allows for the surface chemistry to be changed by placing specific functional groups with controllable surface density and uniform coverage. This feature sets surface-modification using SAMs apart from the wet chemical method.[22]

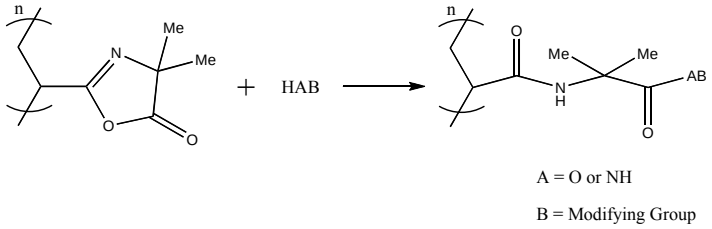
Other widely practiced strategies for modifying a solid substrate with polymers are the "grafting to" approach, "grafting from" approach, and physisorption. Physisorption is defined as a reversible process, while "grafting to" and "grafting from" methods imply chemical bonding between the polymers and surfaces, and therefore are irreversible. The "grafting to" approach is described as reacting end-functionalized polymers with a surface. An alternative to this method is "grafting from" in which initiators are immobilized onto a surface for subsequent growth of the polymers via an appropriate polymerization. The "grafting to" and physisorption approaches allow for only a small amount of polymer to be attached to a surface because the chains have to diffuse through

an existing polymer layer to reach attachment sites on the substrate. The polymer layers produced by these methods are typically characterized by a low grafting density and a small layer thickness.[23] The "grafting from" approach is often more desirable in most cases than the "grafting to" approach because it creates a surface with a higher grafting density.[23] A disadvantage of this strategy is the laborious nature of the immobilization of the initiator on the surface. Additionally, the side reactions during immobilization of the initiator can lead to complications with the multi-layer formation.[23] The ''grafting-from'' technique also suffers problems because of the limitations experienced with initiator efficiency and the effects of side reactions.[24] A noted value of initiator efficiency in literature is 10%.[24] Characterization of the initiator layer is often difficult, making it hard to verify completion of the initiator immobilization reaction, and obtaining the density of initiators that initiated polymerization is problematic.[23] Physisorption is accomplished by the self-assembly of amphipillic block copolymers onto a solid substrate. Selective solvents drive preferential adsorption of block copolymers. The two major problems with physisorption are the instability experience during the adsorption process and the difficulty of synthesizing copolymers for this strategy.[23]

Immobilization of Biomolecules via Azlactone Groups

A monomer gaining recent attention is 2-vinyl-4,4-dimethylazlactone (VDMA).[25] VDMA is attractive because it provides a high conversion of monomer to polymer. Because VDMA can be readily copolymerized, polymers designed with spacing between attachment sites can be made.[25] Scheme 1.3 shows the reaction of VDMA with a

modifying group containing either a primary amine or alcohol end group. The azlactone ring of VDMA is susceptible to nucleophilic attack, and as primary amines are good nucleophiles, they react easily with the azlactone ring, creating a stable covalent amide (CONH) bond. This functionality implies that pVDMA can be attached to a surface and used for the non-selective covalent attachment of biomolecules. Since most biomolecules (proteins, enzymes, DNA, etc.) have an easily accessible primary amine,[9] surface coatings based on pVDMA may be a great resource in applications that require the immobilization of biomolecules. It should also be noted that primary alcohols and water are not good nucleophiles.



Scheme 1.3. Reaction of VDMA with a nucleophilic molecule.

CHAPTER 2

MATERIALS AND EXPERIMENTAL METHODS

Materials and Preparations

Silicon wafers (1 cm × 1.2 cm) were purchased from Silicon Quest and cleaned immediately before use by immersion for 60 minutes in a piranha acid solution (3:1 v/v solution of sulfuric acid (EMD, 95-98%) and 30% hydrogen peroxide (VWR, 29-32%)) followed by rinsing with copious amounts of distilled, de-ionized water and drying with a stream of dry nitrogen. *Warning: piranha acid is a strong oxidizer and should be used with care; as a precaution, organic solvents should be cleared from a hood where piranha acid is being used.* Methyl ethyl ketone (MEK) (Fisher, ≥99%), anhydrous ethanol (Fisher, 99.5+%), and tetrahydrofuran (Burdick and Jackson, ≥99%) were used as received. Poly(glycidyl methacrylate) (PGMA) was prepared via free radical polymerization following the procedure of Luzinov et al.[14] A number-average molecular weight (M_n) of 24,600 g/mol (relative to polystyrene standards) and polydispersity of 1.61 was determined using size exclusion chromatography (SEC) using a Waters Alliance 2695 Separations Module equipped with three Polymer Labs PLgel 5mm mixed-C columns (300 x 7.5 mm) in series and a Waters Model 2414 Refractive Index detector. Dansylcadaverine (Fluka, ≥99%) and 1,6-diaminohexane (Fluka, ≥99%) were used as received. The chemical structures of the main materials used in the preparation of the polymer scaffold are shown in Figure 2.1.

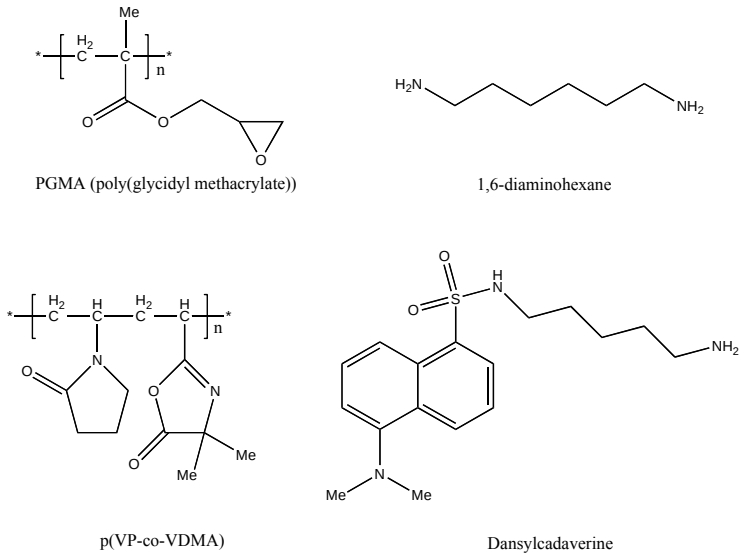


Figure 2.1. Chemical structures of each component of the attachment procedure.

A random copolymer p(VP-co-VDMA) was made via free radical polymerization using AIBN-initiated polymerization of vinyl pyrrolidone (VP) and 2-vinyl-4,4'-dimethylazlactone (VDMA). VP and VDMA were dissolved in benzene at a feed ratio of 3:1. The monomer concentration was 30% weight-to-volume in solvent benzene, and AIBN was added at 2.5 wt.% based on the total monomer mass. The polymerization was conducted at 60°C for 1 hour and the polymer was recovered by precipitation into cold hexanes. Full details on the synthesis and molecular characterization of this copolymer is reported in Macromolecules.[26]

Surface Modification Using p(VP-co-VDMA)

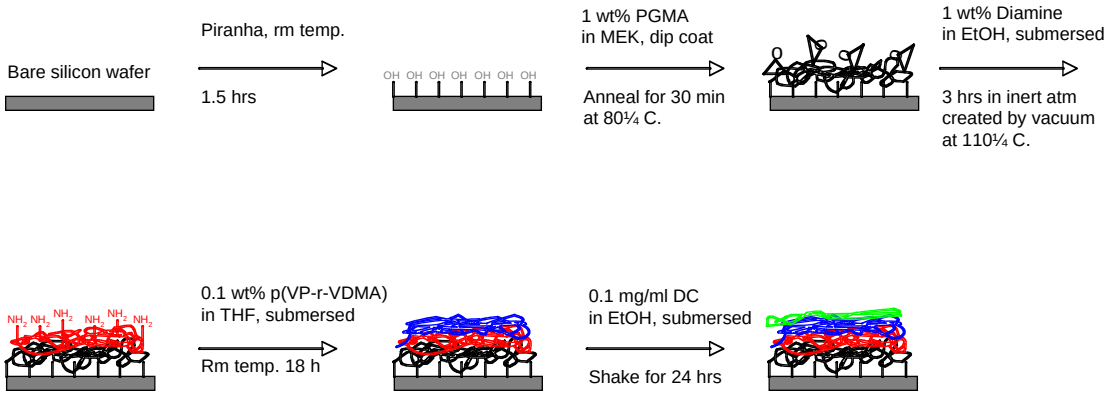
Scheme 2.1 outlines the sequence of steps used to tether p(VP-co-VDMA) to silicon surfaces. It should be emphasized that many different experiments were conducted to evaluate the layer assembly procedure. The specific steps and conditions described here were determined to be the optimum. Each silicon wafer is cleaned by immersion in freshly made piranha solution with the shiny side facing up for 90 min - no external heating of the acid was done. After carefully removing a wafer from the piranha solution, it is washed with copious amounts of distilled water and dried using a filtered nitrogen stream. The samples are prepared immediately before the attachment reactions are conducted. Ellipsometry and contact angle measurements are done between each step of the procedure.

The PGMA attachment procedure is carried out by preparing a fresh 1 wt% solution of PGMA in MEK. The silicon wafers are dip-coated in the solution and annealed in a preheated oven at 110°C for 30 min.[27] When the wafers are cooled to room temperature, they are immersed in MEK and sonicated for 30 min to remove any unattached PGMA from the surface of the wafer, then dried under a filtered N_2 flow.[27]

The PGMA-modified silicon wafers are then submersed in a freshly made 1 wt% solution of 1,6-diaminohexane in EtOH. The wafer and solution are placed in a preheated vacuum oven at 80°C for 3 hours utilizing the vacuum to create an inert atmosphere. The wafers were sonicated for 30 min in EtOH after they had cooled to room temperature, and then they were dried with a filtered N_2 stream.

In order to attach the p(VP-co-VDMA) to the diamine layer, the wafers are immersed for 18 hours in a freshly made 0.1 wt% solution of p(VP-co-VDMA) in THF. After the allotted time had passed, the wafers were removed from the solution and sonicated for 30 min in THF before being dried with a stream of filtered, dry N_2.

The fluorescent molecule dansylcadaverine was attached by immersing the wafer in a 0.1 mg/ml solution of dansylcadaverine in EtOH and shaking it for 24 hours. The wafers were removed from the solution, rinsed with EtOH, and dried under a flow of filtered N_2.



Scheme 2.1. Schematic of fluorescent molecule attachment to silicon wafer.

The schematic above demonstrates the rationale of my process: PGMA robustly anchors to silicon, but leaves many available epoxide groups. Diaminohexane reacts with the epoxide groups, but leaves other primary amine groups free at the surface. Control experiments aimed at optimizing the diamine attachment process are given in Appendix

B.1. These free amines are used to anchor p(VP-co-VDMA) onto the surface. Some azlactone groups remain available for subsequent reaction so that dansylcadaverine (DC) can be grafted onto the surface.

Characterization

Fourier Transform Infrared Reflectometery (FTIR)

The transmission spectra were obtained from a Bruker Optics Vertex 70 at the Center for Nanophase Materials Sciences at Oak Ridge National Laboratory by Dr. Jamie Messman. The IR beam was transmitted directly through the silicon. The background used was a spectrum of a bare silicon wafer. The aperture setting was at 6 mm, with KBr as the beamsplitter and the DTGS detector. The scanner velocity was 10 kHz. The Fourier Transform parameters used are as follows: Blackman-Harris 3-Term apodization function; phase resolution of 32 cm^{-1}; phase correction mode of Mertz; zero filling factor of 2. The acquisition parameters used were 254 scans at a resolution of 4 cm^{-1} with 512 background scans.

Ellipsometry

Thickness measurements were made using a variable angle Beaglehole Picometer Ellipsometer, which utilizes a He-Ne laser light source (λ=632.8 nm). Measurements were conducted at integer values of the incident angle, ranging from 80° to 35°. The thicknesses reported are averages of three ellipsometric measurements from selected areas near the center of a modified silicon wafer. The refractive indices used for each

layer are as follows: η_{PGMA} = 1.525,[28] $\eta_{Diamine}$=1.5, $\eta_{p(VP\text{-}r\text{-}VDMA)}$=1.5, η_{DC}=1.5. A model based on a uniform layer was created for each step of the attachment procedure (each strata shown in Scheme 2.1) was used to analyze the data. Because the average thickness of each strata was obtained after it was formed, those values were used (not allowed to vary) when determining the thickness of a subsequent layer in the multilayer film.

Contact Angle

Static water contact angles were measured using the sessile drop method with a Krüss DSA10-Mk2 instrument and digital photo analysis software. A water droplet (volume ≈ 1 μL) was placed onto the wafer by automatic dosing using a syringe. The contact angle values reported are averages of three drops along the center of a modified silicon wafer. When the measurement was complete, the sample was dried with a steady stream of filtered N_2.

Fluorometry

A PTI (Photon Technology International) QuantaMaster UV-VIS spectrofluorometer was utilized to determine the fluorescence of the modified silicon wafer after dansylcadaverine attachment. Samples were placed at a right angle to the incident beam with excitation and emission wavelengths at 380 nm and 450 nm, respectively.

X-ray Photoelectron Sectroscopy

XPS analyses were performed on a Thermo Scientific K-Alpha XPS utilizing monochromatic Al K_α x-rays. The x-ray spot size was 400 microns and the samples were analyzed with an automated charge compensation device that uses both low energy Ar ion and low energy electrons. Samples were introduced into the analysis chamber via vacuum load-lock and were analyzed without any other preparation. Survey spectra were obtained (0-1300 eV) to check for unexpected contamination. Narrow region energy scans were made for C 1s, N 1s, O 1s, S 2s, and Si 2p (substrate) to determine composition.

Drop-on-Demand Biomolecule Attachment

Drop-on-Demand (DOD) inkjet printing uses printhead nozzles to release a single drop of ink precisely onto a surface to form any type of pattern. A collection of these drops, which construct what is known as pixels, on the surface creates the desired patterned result that our eyes interpret as an image. In this research, a gradient pattern was used. The volumes of the small drops that exit from the inkjet printer are generally on the order of picoliters. The resolution of a printer denotes the distance between two adjacent ink drops, but the dissimilarity between printed pixels actually defines the pattern resolution. This feature gives the printer the ability to make pronounced visual graphics. Inkjet printing allows one to use small drop volumes of liquid solutions, experience high printhead operating frequency, remarkable system reliability, and extremely regulated ink drop placement.[17]

As described in our previous publication,[17] a Canon BJC-series printer was modified slightly by removing the casing and a few feeding rolls to accommodate feeding of the substrates. In order to resolve any differences in the solution viscosities of traditional inks and ethanol solutions containing dansylcadaverine, the print drivers were modified as noted by Pardo et al.[29] The printer resolution used was 720 (vertical) x 360 (horizontal) dots-per-inch (DPI) maximum. Microsoft PowerPoint was used to design the gradients consisting of a two color blend from black to white and patterns. Pattern guides (used to locate the silicon chips in the proper position on the page so the pattern is printed onto them) were printed on transparencies with black ink. A 0.1 mg/ml solution of dansylcadaverine in ethanol was prepared in advance and injected into a previously cleaned ink tank and installed in the printer.[17] A silicon substrate modified with a p(VP-co-VDMA)-capped multi-layer (using the attachment strategy previously described) was secured to a pattern guide (transparency) with double-sided tape. After the dansylcadaverine pattern was printed onto the sample, the ethanol was allowed to evaporate before the sample was disengaged from the pattern guide.

CHAPTER 3

RESULTS AND DISCUSSION

p(VP-co-VDMA) Functionalized PGMA-modified Surfaces

A multilayer scaffold was used to tether p(VP-co-VDMA) copolymers to the surface. In the first step, PGMA was deposited onto the silicon substrates via dip coating from a 1.0 wt% solution of PGMA in MEK. This is shown in step 1 of Figure 3.1. After the volatile solvent evaporated, the films were annealed for 30 min under vacuum in an oven preheated to 110°C. After the PGMA-modified surfaces were removed from the oven, they were sonicated in MEK for 30 min in order to remove any non-bonded chains. Dry layer thicknesses measured by ellipsometry were consistently between 12 and 20 nm. Liu et al. have previously demonstrated[14] this approach for creating a "primer" polymer layer, permanently attached to the substrate that can be used to anchor other species to interfaces. Furthermore, they showed that this approach was reproducible, yielding smooth and uniform films, and that the PGMA layer thickness is proportional to the concentration of PGMA in MEK solution. They used a low molecular weight substance as a probe for the presence of the accessible epoxy groups and reported that after annealing at 110°C for 30 min, approximately 40% of the epoxy groups were still available on the layer.[14]

Figure 3.1 displays the results from ellipsometric measurements of samples obtained after each step of the layer attachment process. The protocols used to modify each sample along each step of the attachment process were held constant. Sample 1 was

carried out to through the attachment of PGMA; sample 2 through the diamine attachment; sample 3 through the p(VP-co-VDMA) attachment; and sample 4 through the biomolecule attachment. The PGMA thicknesses were around 6 nm, which is consistent with expectations when a 1 wt% PGMA solution is used.[14] In agreement with results from Zdyrko et. al.,[30] a static water contact angle of approximately 60° was measured for the PGMA on the silicon surfaces. The reaction of epoxy groups with primary amines has been studied extensively.[31] When an epoxy group reacts with a primary amine, a secondary amine and secondary alcohol are formed. In my case, if one end of the diamine reacts, the other is available for the easy attachment of the p(VP-co-VDMA) polymer. The diamine addition raised the overall thickness of the sample approximately 7 +/- 1 nm. The thickness increase suggests that the diamine readily penetrates and reacts throughout the PGMA Layer. The contact angles measured for the diamine-functionalized layer was approximately 53°, which is consistent with the value of 56° reported for the water contact angle of amines.[29]

The pendant azlactone ring of the VDMA moiety reacts with an amine group through nucleophilic addition with no byproducts.[32] This reaction forms a covalent bond yielding a stable amide linkage.[4,10-11] Addition of the p(VP-co-VDMA) increased the overall thickness by about 2 nm +/- 0.5 nm, and water contact angles of 66° were measured. The thickness added by the p(VP-co-VDMA) attachment is consistent with the notion that, as opposed to the small molecule diaminohexane, which penetrates and attaches throughout the previous layer, the high molecular weight copolymer attaches only at the interface of the film. When dansylcadaverine was allowed to react with the

p(VP-co-VDMA) layer, the thickness increased only ~1.3 nm. As shown in Figure 2.1, dansylcadaverine has a single primary amine, and it attaches to the azlactone ring via nucleophilic addition (i.e., Scheme 1.3).

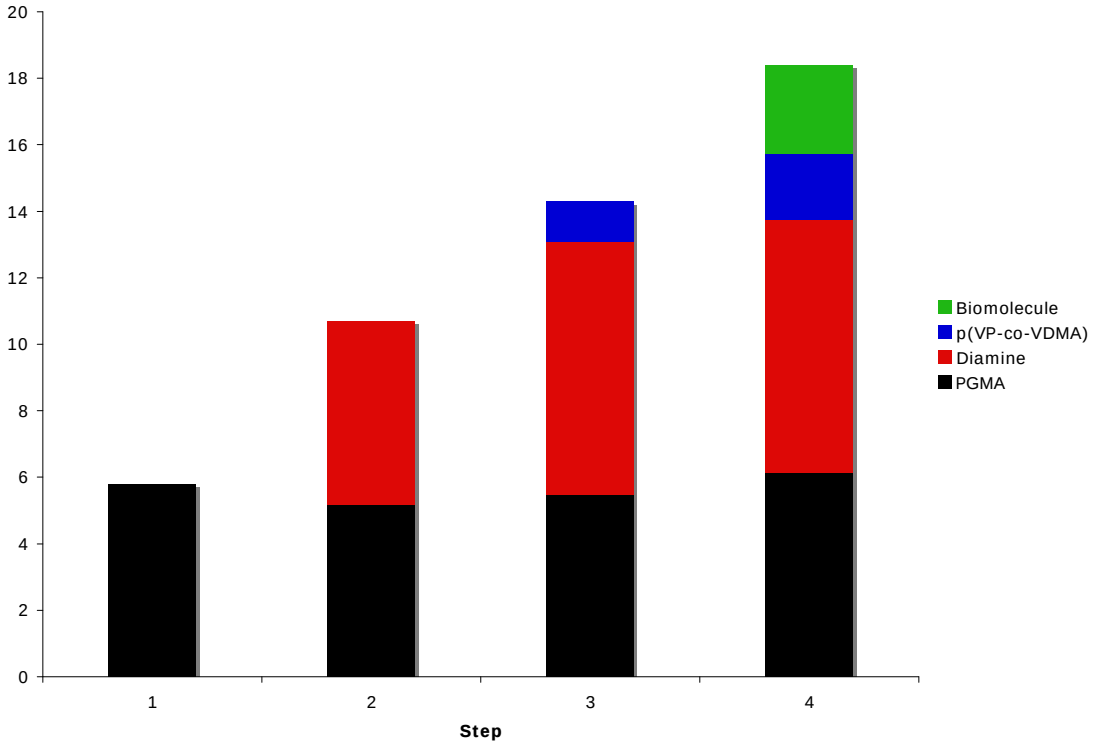


Figure 3.1. Ellipsometric thicknesses of each step in the preparation of multilayer scaffold.

Spectroscopic Characterization

Figure 3.2 shows the results from FTIR characterization after each step of the attachment procedure. The results are used to prove the existence of the expected bond

stretches that are present after each step of the procedure. The sample preparation of JEB-1 was stopped after the attachment of PGMA. Sample JEB-2 was carried out through the reaction with the diamine, and JEB-3 continued through the p(VP-co-VDMA) attachment. As previously noted, all samples carried through a given step were reacted under identical conditions. From the IR results, we can be sure that the PGMA was present in all of the samples because of the peak at 1230-1260 cm^{-1} and 1720 cm^{-1}, which are due to the C-O stretch of the ester and the C=O stretch found in PGMA. The broad peak in JEB-1 at 1150 cm^{-1} is attributed to the C-O-C asymmetric stretch of the unreacted pendant epoxides. The chemical structure for PGMA can be found in Figure 2.1. XPS results shown in Table 3.1 are also consistent with PGMA attachment. The C/O ratio expected for PGMA is 2.33, and a value of ~2.09 was measured for the JEB-1 sample. Undoubtedly, some of the discrepancy arises because some of the oxygen signal results from the native oxide layer. In addition to the ellipsometry results, the XPS results also show that the scaffold is increasing in thickness after each step of the attachment procedure. Specifically, the C/Si ratio increases from sample JEB-1 to JEB-3, indicating that the layer is getting thicker and fewer electrons from the substrate are reaching the detector.

The reaction of the PGMA layer with the diaminohexane is shown in Scheme 3.1. In this reaction (resulting in sample JEB-2), the diamine produces an alcohol that can be seen in the C 1s scan Figure 3.3. Also, the N 1s scan (Figure 3.4) shows the appearance of the C-N in the JEB-2 sample. The C/N ratio for the diamine-modified PGMA from Table 3.1 is ~11.6. If the diamine attachment reaction is assumed to be 50% efficient, the

C/N ratio should be 10. Assuming a 40% efficiency, the C/N ratio should be ~11.8, and a 41.1% efficiency gives a ratio of 11.6. From this, it can be estimated that the efficiency of the diamine reaction is around 41%. Zdyrko et. al.[33] reports that the annealing of the PGMA layer leaves approximately 40% of the epoxy groups available for further attachment, so it can be said that the amine attached onto all active epoxy groups.

The pendant epoxide peak evolves into two peaks in the IR spectra when the epoxy groups are reacted with the diamine. One group remains at 1150 cm^{-1}, which is likely due to the unreacted epoxy groups. The other peak appears at 1113 cm^{-1}, which is seen because of the C-N stretch of amines. This mode occurs because, as mentioned previously, the reaction of the epoxide with a primary amine yields a secondary amine and a primary alcohol. This mode at 1113 cm^{-1} is present in both JEB-2 and JEB-3. According to Silverstein et al.,[34] the C-N stretch of primary amides occurs near 1400 cm^{-1} and it is very prominent in JEB-3 but almost nonexistent in JEB-2. The pendant lactam C(=O)-N present due to PVP causes a peak to appear at 1285 cm^{-1} in JEB-3. Also, the C=O carbonyl stretch for PVP is centered at 1680 cm^{-1}, which overlaps the amide I band that is created when p(VP-co-VDMA) is reacted onto JEB-2. This reaction scheme can be found in Scheme 3.2. The amide II band, typically at 1650 cm^{-1}, is seen as a shoulder on the peak centered at 1680 cm^{-1}. The N 1s scan (Figure 3.5) of the JEB-3 sample shows the clear appearance of the amide expected when an azlactone ring is opened by a primary amine. Also showing the success of the coupling of the diamine group onto PGMA (JEB-1) is the emergence of N signal in JEB-2 and JEB-3. The C/N ratio for JEB-3 is ~9.1, which corresponds with an assumed ~67% efficiency for the p(VP-co-VDMA)

reaction. The C/N ratio is decreasing with each step, as it should because of the addition of the diamine and then addition of p(VP-co-VDMA). Each of these attachments gives rise to N 1s signal, but also adds more C 1s due to the high carbon content of the diamine and copolymer.

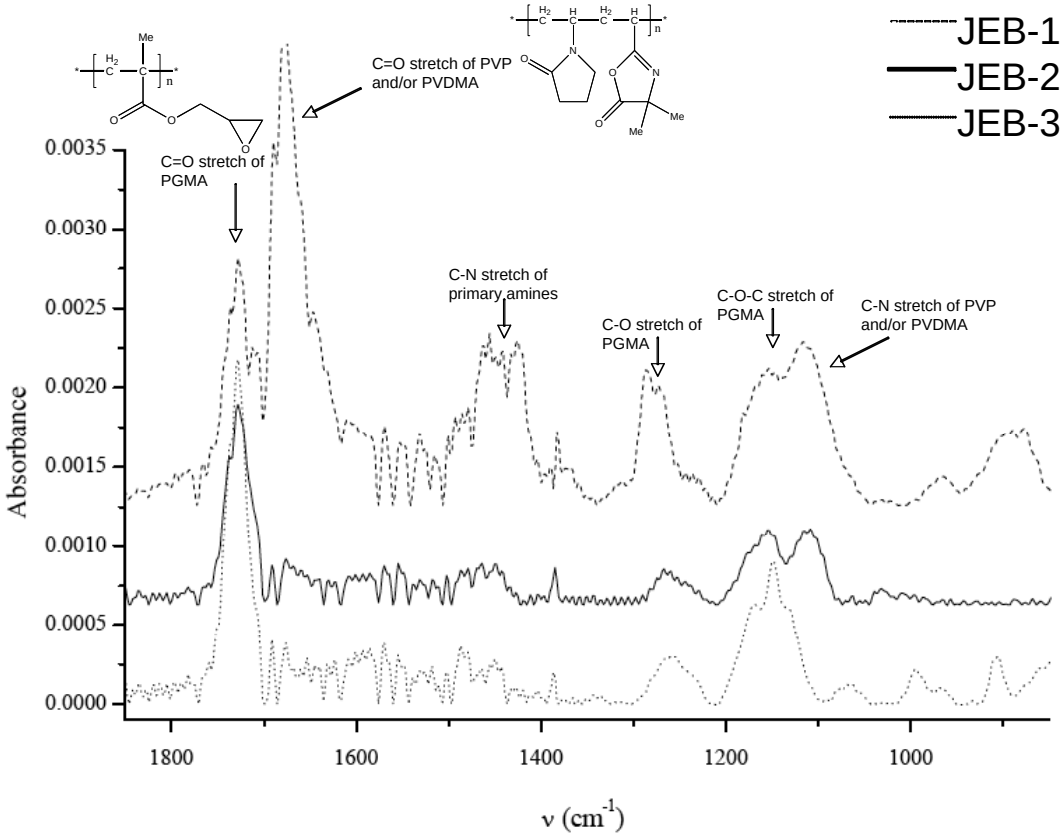
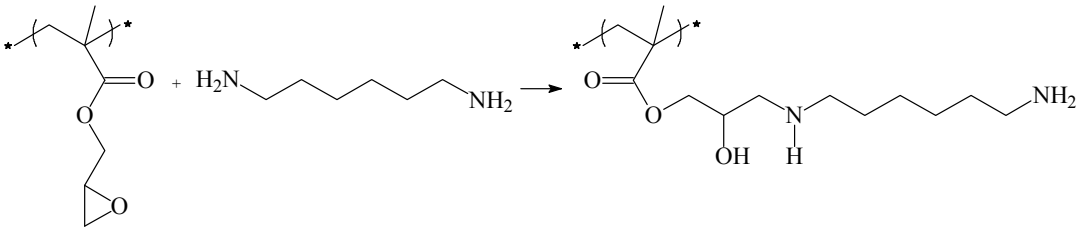


Figure 3.2. FTIR spectra showing chemical changes occurring along the progressive steps of the attachment procedure.

Table 3.1. Atomic composition of samples as obtained from XPS measurements.

Atomic Composition (at.%)				
Sample ID	**C**	**O**	**N**	**Si**
JEB-1	60.0	28.7	0.0	11.3
JEB-2	64.7	22.7	5.6	6.7
JEB-3	70.7	15.7	7.8	5.4



Scheme 3.1. Propsed result from reaction of PGMA layer with 1,6-diaminohexane, yielding a diamine-modified PGMA.

JEB-2		
Element	**Peak BE**	**At. %**
C-C and C-H	284.9	38.3
C-N (amine)	286.0	5.5
C-O-C and C-O-H	286.5	11.4
C(=O)-N (amide)	287.7	3.0
O-C=O	288.7	6.5

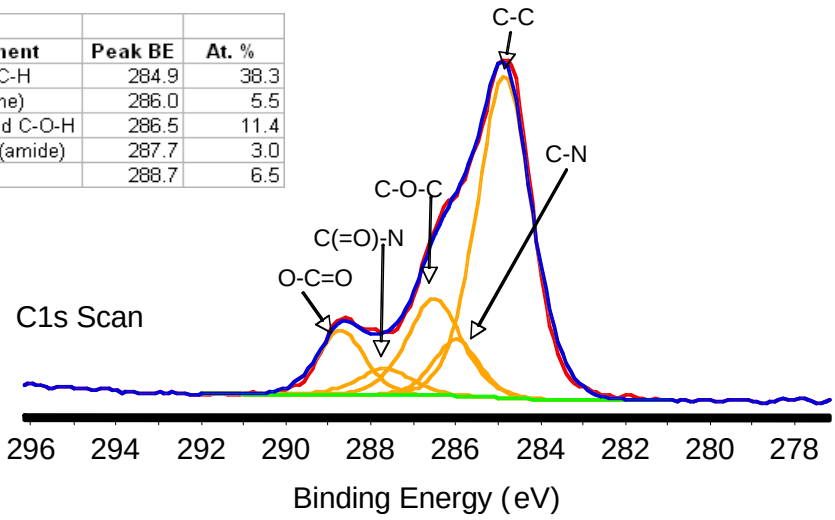


Figure 3.3. C 1s scan of sample JEB-2.

JEB-2		
Element	**Peak BE**	**At. %**
C-N (amine)	399.6	4.2
C(=O)-N (amide)	401.4	1.4

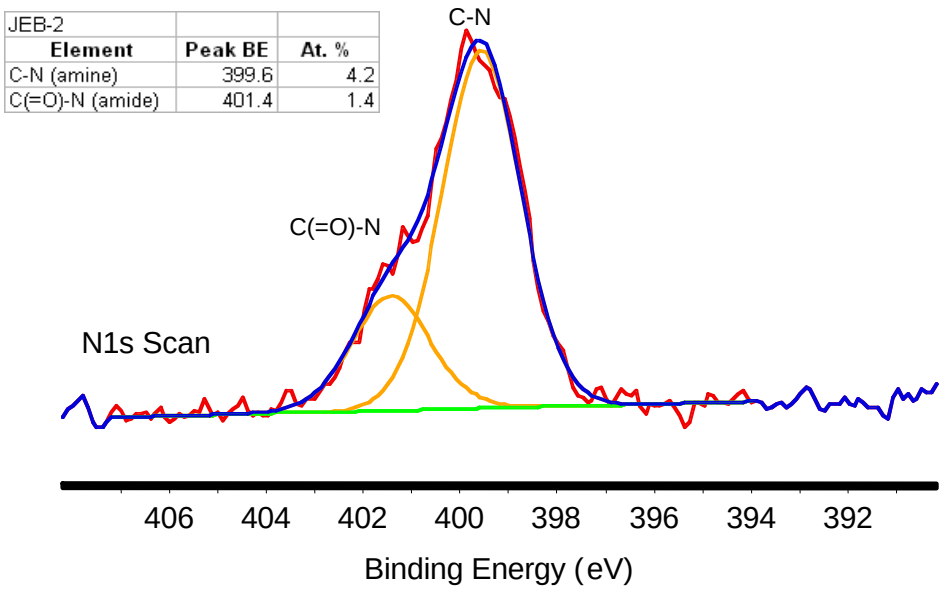
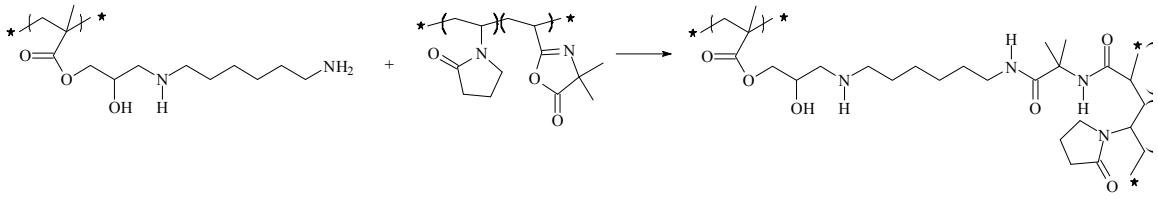


Figure 3.4. N 1s scan of sample JEB-2.



Scheme 3.2. Proposed reaction of diamine-modified PGMA with p(VP-co-VDMA). Contrary to this simplified schematic, spectroscopic evidence supports the notion that not all of the azlactone groups of the copolymer react with surface-tethered amines.

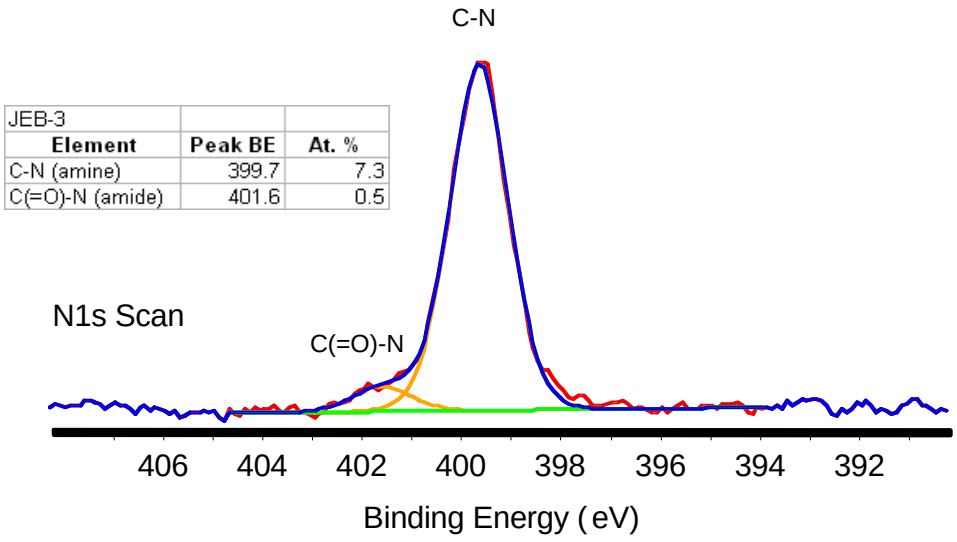


Figure 3.5. N 1s scan of sample JEB-3.

Biomolecule Attachment and DOD Printing

After the successful construction of the multi-layer scaffold, the biomolecule attachment was investigated. Because dansylcadaverine is fluorescent,[35] its attachment can be verified by the use of a PTI (Photon Technology International) QuantaMaster UV-VIS spectrofluorometer. Figure 3.6 shows the fluorometry plots for the steps of the modification process. Scheme 3.3 shows the expected product of the reaction of the scaffold with dansylcadaverine.

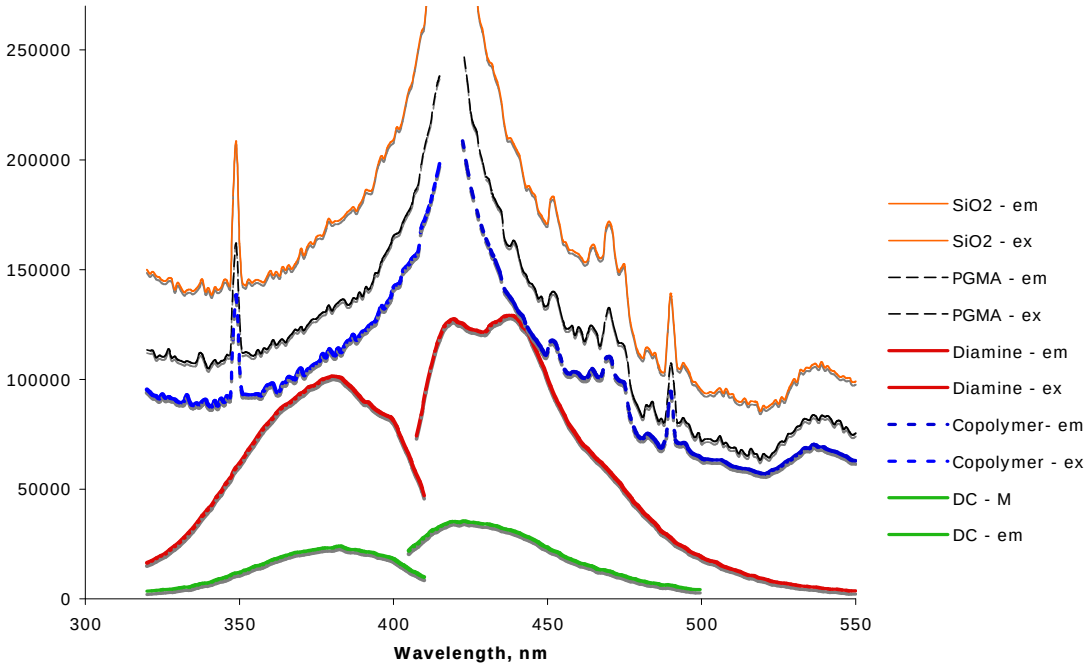
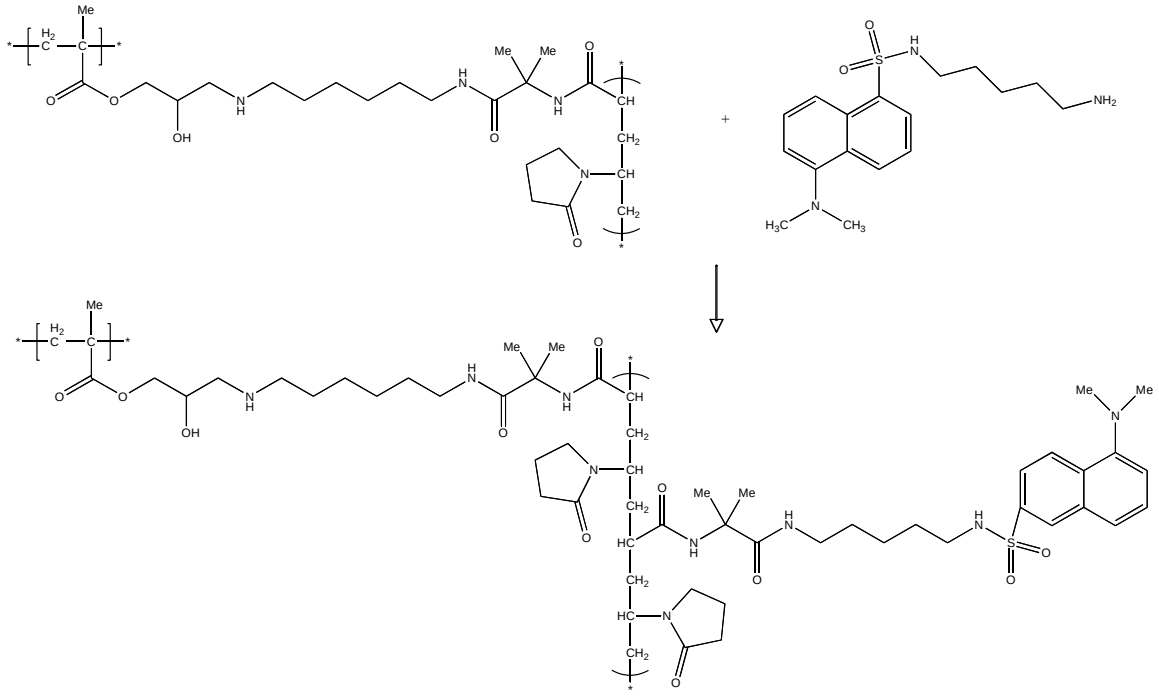


Figure 3.6. Fluorometry measurements plotted for each step of the PGMA-modified surface procedure. (*Note: "em" designates the emission curves (right side), while "ex" designates the excitation curves (left side).)*



Scheme 3.3. The result of the reaction of dansylcadaverine with the PGMA-modified scaffold.

A blank sample consisting of a cleaned silicon wafer (labeled SiO2 in the legend of Figure 3.6) displayed no fluorescence, as expected. This particular excitation and emission trend (as seen in Figure 3.3) will be called a “dead curve” because there is no fluorescence intensity. The featureless dead curve provides a benchmark that allows us see which layers fluoresce. Even though this trend is referred to as “dead”, peaks are noticed at 350 nm on the excitation curve and between 450-490 nm on the emission curve – these are attributed to the lamp being reflected to the detector since the surface is shiny.

They do not indicate any fluorescence. Because the lamp is more intense than emitted fluorescence, the dead curves will be higher in intensity than a fluorescence curve.

A surface modified with PGMA only also exhibits this so-called dead curve behavior, but is not in the exact same position (it does not overlap, it is shifted vertically). It is known that any change in the structure of a surface will cause the curve to shift, so this shift in reflectance provides more evidence that the PGMA is attached. The diamine, however, fluoresces, and therefore a diamine-modified PGMA fluoresces. As can be seen in the emission curve labeled "Diamine", there is a broad maxima around 380 nm with the excitation maxima at approximately 420 nm and 440 nm. According to Ichinose et al.,[36] the excitation spectra for primary and secondary amines have maxima at 425-430 nm and emission maxima at 475-480 nm. This near-60 nm spread between maxima is noticed in the diamine fluorometry results, but the wavelengths are shifted to slightly lower wavelengths. Visual observation of the sample showed the emission of the expected brilliant blue light. This light emission is expected because, as the aforementioned XPS results displayed, the surface of the sample is covered with amine groups. This coverage allows the light emission contribution to be greater than the light that is being reflected by the surface, thereby producing a luminescent profile.

After the p(VP-co-VDMA) is reacted with the diamine-functionalized scaffold, the fluorescence curves return to the dead curve behavior. This, along with the vertical shift helps support the presence of the polymer layer and its binding with residual primary amines, as the copolymer layer should not be displaying any fluorescence. Since we know that this reaction was carried out with ~67% efficiency, this leaves roughly 30%

of the amine groups unreacted with the copolymer. Because of this, the reflective contribution of the sample far outweighs any fluorescence that could be detected, thus producing the dead curve profile with no apparent luminescent properties. Once dansylcadaverine was allowed to react with the p(VP-co-VDMA) layer, the fluorometry measurements show the sample to be exhibiting fluorescence again, proving that dansylcadaverine was successfully attached to the modified silicon surface. The signal is significantly weaker than the diamine fluorescence signal (see Figure 3.4), which corresponds with the surface coverage of dansylcadaverine as compared to the diamine. Dansylcadaverine is known to have an excitation wavelength of 340 nm,[16] which is within reason of the ~380 nm that I found during my fluorometry measurements.

Given the success demonstrated with the attachment of a model biomolecule onto the multi-layer surface scaffold, preliminary attempts to use DOD inkjet printing as a means of depositing DC onto p(VP-co-VDMA)-modified scaffolds. A 0.1 mg/ml solution of dansylcadaverine in ethanol was prepared in advance and injected into a previously cleaned ink tank and installed in the printer. A few test pages were printed prior to the printing of the modified substrate. The modified silicon substrate was secured to a pattern guide (transparency) with double-sided tape. The pattern chosen for printing was a two-"color" gradient from white to black, where black is the only trigger for ink to be ejected onto the surface. Here, color corresponds to the dansylcadaverine concentration. After the gradient dansylcadaverine was printed on the p(VP-co-VDMA)-modified surface, the ethanol was allowed to evaporate before the sample was removed from the pattern guide sheet. Ellipsometric measurements were made on the graded, dansylcadaverine-printed

scaffold. The results show that one end of the modified silicon substrate had a dansylcadaverine thickness of 0 nm, an area near the midpoint of the sample showed a thickness of 1.4 nm, and at the end of the substrate where the gradient was richest in printed DC, the thickness was 8.1 nm. These results indicate that a gradient of DC was printed onto the underlying p(VP-co-VDMA)-modified surface; however, it should be noted that the calculated thicknesses of DC are based on the assumption that the underlying scaffold thickness is uniform. While the previous results show that sample-to-sample variations in PGMA thickness of 12-20 nm may occur, the variations in PGMA layer thickness in any given sample was much smaller, typically 1-2 nm.

A longer sample modified with the multilayer scaffold (shown in Figure 3.7a) was subjected to DOD printing with a gradient. (The irregular shape of the sample is due to the position on the precut silicon wafer from where this sample was removed.) Shown with the sample is the gradient, with darkened shading of the gradient corresponding to where the DC-containing “ink” is printed. A line scan of the sample was taken by XPS as shown in Figure 3.7b; the line consisted of 20 equally spaced points from one end to the other across the sample, perpendicular to the graded direction. From Figure 3.8, one can see that there is a uniform composition in this direction across the sample, which means that the sample had equal coverage of the DC-ethanol solution in the y-direction.

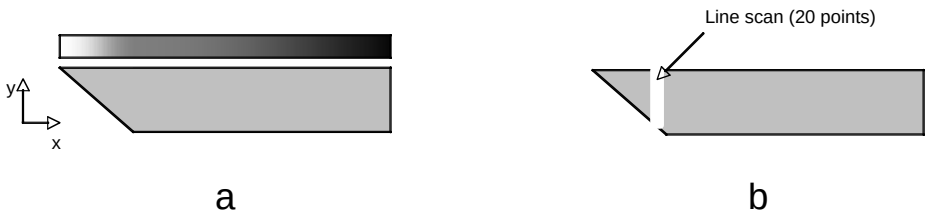


Figure 3.7. (a) Sample used for DOD printing the biomolecule. (b) Indicated line is placement where XPS line scan measurements shown in Figure 3.8 were made.

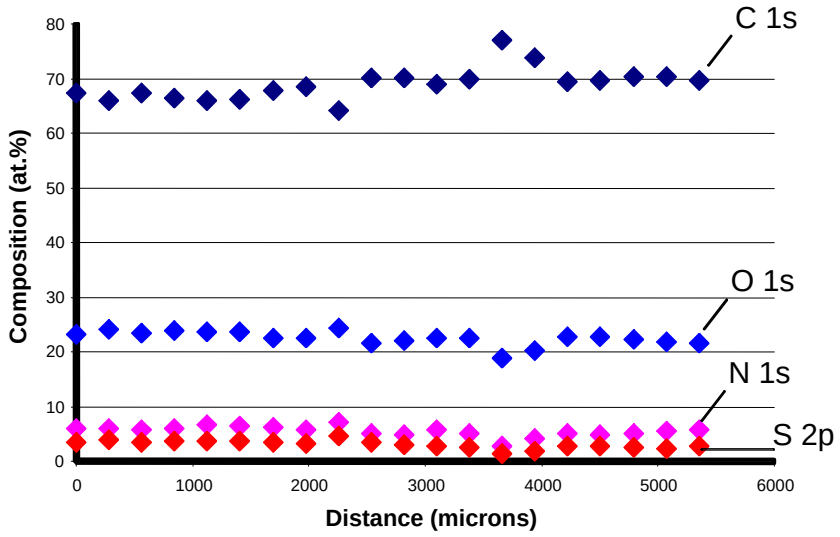


Figure 3.8. Composition of C, O, N, and S scans from the line scan shown in Figure 3.7b.

Next, an attempt was made to map the atomic composition along the gradient direction, as shown in Figure 3.9. The data collected is from a grid of 15 points in the dimension across the gradient by 50 points in the direction along the gradient. The 750 individual points were grouped together into 25 slices, each containing 30 points (except slices 1 and 2 at the narrow end of the sample, which contain 26 and 28 points, respectively). From each slice, the average C, O, N, S, and Si spectra for the region were computed. As seen in Figure 3.10, the slice number increases (moving along the x-direction), the C 1s/ Si 2p increases, reflecting an overall increase in thickness of the layer along the gradient direction. The C 1s/ S 2p gives an overall decreasing trend. This decrease would be expected as only one S atom is present in DC, which contributes significantly more C atoms when attached to the surface.

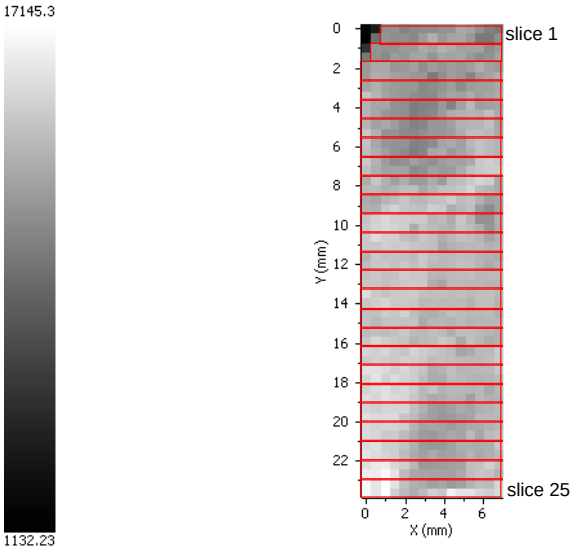


Figure 3.9. Map of the sample showing the layout of how the points are grouped together.

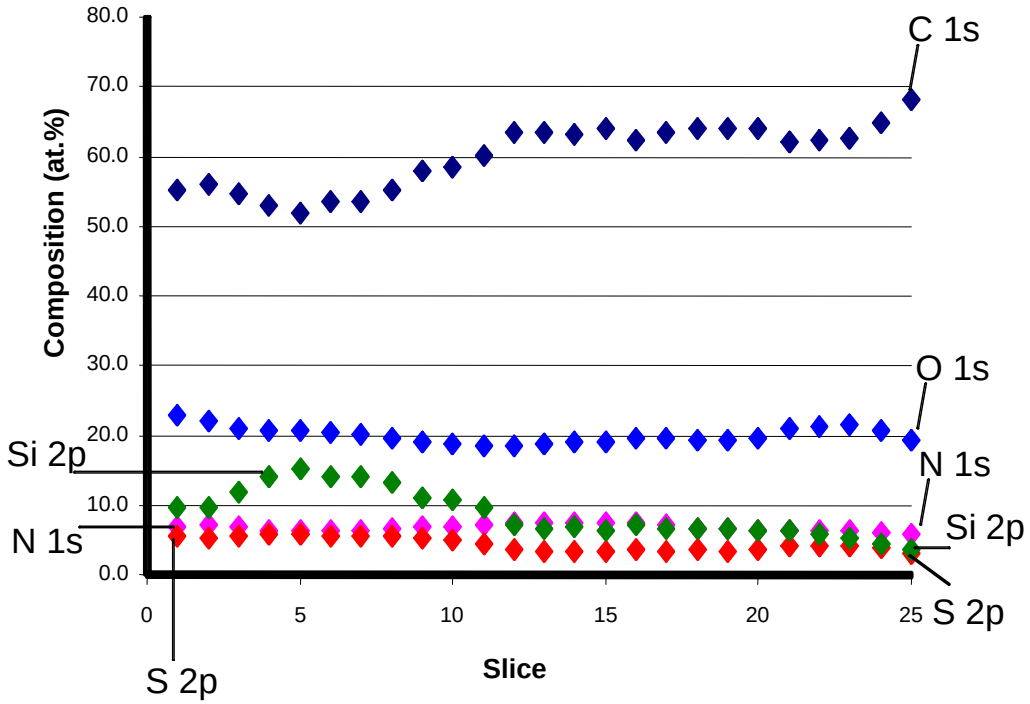


Figure 3.10. Plot showing the average composition in each slice.

Although no attempts were made to optimize the DC DOD printing process, these preliminary results indicate the feasibility of the approach and overall promise of VDMA-containing surface scaffolds as novel biomaterial coatings. With the success in constructing the multilayer scaffold, the DOD inkjet printing method of attaching a biomolecule was explored. With the spectroscopic and ellipsometric results, the DOD printing study was also successful. The results obtained from XPS gave substantial evidence to the existence of the anticipated chemical structure of the surface. With this multilayer scaffold and printing technique, multiple designs could be used to attach a biomolecule.

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

A novel procedure for the immobilization of biomolecules onto a multilayer polymer scaffold was explored, and the preparation of such a surface scaffold that can be used for non-specific binding of biomolecules was successfully carried out. The surface layers formed after each step of the procedure were characterized via FTIR, ellipsometry, fluorometry, and XPS. The procedure implemented led to the successful formation of a multi-layer polymer scaffold with easy repeatability. The physical and chemical characterizations verified the completion of each step of the process. The VDMA-based polymers are advantageous because of their hydrolytic stability and pendant azlactone rings undergo nucleophilic attack without byproducts to form stable covalent bonds with primary amines. An example is functionalized with dansylcadaverine. The nucleophilic addition reaction proceeded without a catalyst and at room temperature, yielding a stable amide linkage. This feature adds to the ease of construction expected when using VDMA-based polymers. After the attachment of biomolecules onto the modified silicon surface was proven to be successful, then the attachment via DOD inkjet printing was explored. Because of the flexibility and ease in pattern design, DOD inkjet printing is an interesting method for creating surfaces with one or more patterns of biomolecules, though additional research is needed to identify proper conditions for attachment.

Recommendations for future work include optimizing the attachment procedure of the biomolecule dansylcadaverine: in addition to exploring the reaction time and temperature, the concentration of the dansylcadaverine solution also should be investigated. With respect to the DOD inkjet printing of the biomolecule, different patterns need to be tried in order to further the understanding of how biomolecules can be printed onto the surface. Optimizing the concentrations used in the reactions of each step of the attachment procedure detailed in this work is worth also worth exploring.

APPENDICES

Appendix A

Experimental Procedures

A1. Sample Preparation

Modifying Silicon Surface with Multilayer Polymer Scaffold

1. Prepare piranha solution in a clean beaker. The solution is created by pouring 30 ml of sulfuric acid in the beaker, then slowly adding 10 ml of cold, 30% hydrogen peroxide to the acid. WARNING: Extreme caution should be taken when adding the hydrogen peroxide, as this is a highly exothermic reaction.
2. Silicon wafers (1 cm × 1.2 cm) are carefully removed from the secure palette and carefully placed in the piranha solution, shiny side up, for 90 min.
3. Immediately after removal from the piranha acid solution, a silicon wafer is washed with distilled water, dried under a filtered N_2 flow, washed with acetone, and dried under a filtered N_2 flow.
4. Prepare a 1 wt% solution of PGMA in MEK. Sonicate the solution to ensure the PGMA is fully dissolved.
5. A silicon wafer is dipped in the solution and allowed to dry under ambient conditions. The wafer is held as flat as possible to create even coverage as the solvent evaporates.
6. After the MEK has evaporated, the sample is placed in a vacuum oven (110°C, 1 atm) for 30 min. Upon removal from the oven, samples are allowed to cool before being sonicated in MEK for 30 min. The sample is then dried under a filtered N_2 flow.

7. Prepare a 1 wt% solution of 1,6-diaminohexane in ethanol. A sample is placed shiny side up in ~3ml of this solution. Note: The amount of solution used for this step is solely dependent on the size of wafer used. The idea is simply to have a sample completely submerged, but without too much excess.
8. This is placed in a vacuum oven with a cold trap (80°C, ~10 mmHg) for 3 hours. The vacuum is used to create an "inert" atmosphere of ethanol which evaporates to create the atmosphere. Note: It is imperative to not let the solution reach a bumping point.
9. Upon removing the sample from the vacuum oven, the sample is allowed to cool before being sonicated in fresh ethanol for 30 min and then dried under a filtered N_2 flow.
10. Prepare a 0.1 wt% solution of p(VP-co-VDMA) in tetrahydrofuran. A wafer is allowed to sit in the solution at room temperature for 18 hours. After the allotted time has been reached, the sample is removed, sonicated in tetrahydrofuran for 30 min, and finally dried under a filtered N_2 flow.

A2. Biomolecule Attachment to Polymer Scaffold

Total Surface Biomolecule Attachment

1. Prepare a 0.1 mg/ml solution of dansylcadaverine in ethanol. Sonicate as needed to ensure that the dansylcadaverine is fully dissolved.
2. A modified silicon surface (as prepared by the procedure described in A1) is immersed in the solution in an Erlenmeyer flask. The flask is shaken for 24 hours.
3. After removing the sample from the flask, it is allowed to dry in ambient conditions.

DOD Inkjet Printing of Biomolecule

1. Prepare a 0.1 mg/ml dansylcadaverine in ethanol solution. Sonicate as needed to ensure the dansylcadaverine is fully dissolved.
2. Inkjet cartridges are cleaned with water by placing a small rubber tube into the inside of the printhead. This allows water to be pushed through and it can be seen exiting as a fine mist. All printheads are cleaned in this fashion; there are three per cartridge.
3. After each head has been cleaned, ethanol is injected into the cartridge. Test printing onto transparencies is done until ethanol is being printed. This may take a few pages.
4. The dansylcadaverine solution is injected into the cartridge. Test pages are run until the solution is being printed.
5. A modified silicon surface (as prepared by the procedure described in A1) is secured to a pattern guide transparency via double-sided tape. The transparency guide is

placed in the paper feed tray and the pattern template in Microsoft PowerPoint is printed.

6. The ethanol is allowed to evaporate before the sample is disengaged from the pattern guide transparency.

A3. Static Water Contact Angle Procedures

Equipment Set-up

1. Turn on the computer and the Krüss DSA10-Mk2 instrument.
2. Open the Drop Shape Analysis (DSA) software on the desktop of the computer.
3. Fill the syringe with desired liquid and secure the syringe in the device above the measured surface.
4. Select "File/Open FG-Window" to obtain a live video image.
5. Adjust the height of the needle using the "Needle Position" tab until it appears at the top edge of the live video image.
6. The focus dial of the camera is used to obtain a crisp image of the needle.
7. Using the software, make a 1 μl drop of liquid by pressing the up arrow. Figure A.1 shows the needle with a drop of water and Figure A.2 shows the control panel.
8. Slowly raise the stage until the sample surface just touches the bottom of the drop. (Note: Watch the live video image as the sample and stage appear on screen. See Figure A.3.)
9. As soon as the drop has been placed on the surface, raise the needle out of the live video image.
10. Using the focus dial of the camera, focus the image.
11. Press the camera icon to capture the image in the FG-Window. This image may be saved and analyzed later.

Figure A.1. Image of needle with a ~1 µl of water droplet.

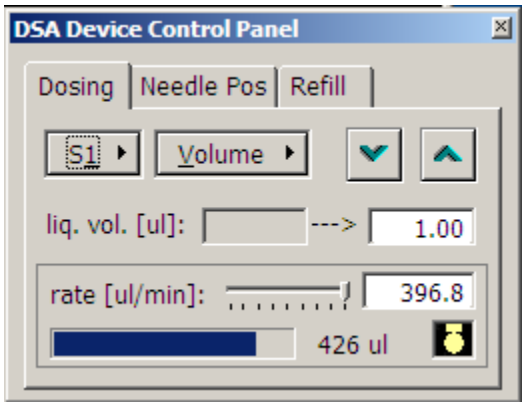


Figure A.2. Control panel for the Drop Shape Analysis software.

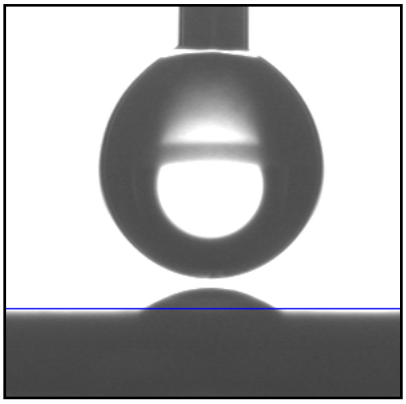


Figure A.3. Image of the sample being raised to the water drop.

Measuring the Static Water Contact Angle

1. Select the contact angle image desired for angle measurements. This may be a previously saved image or the one on the screen.
2. Select the  icon for the software to automatically find the drop-surface interface (the blue line). If the automatically generated baseline does not appear to be correct, adjustments can be made by selecting and moving the "+" found on the left side of the drop. The "+" should be placed at each side of the drop-surface interface. Figure A.4 shows the image with a corrected baseline and "+" for manual correction.
3. Select the  icon to calculate the contact angle.
4. Click the  icon to show the measured angles in the "Data" window. If doing multiple drops per surface, the program will automatically calculate the average and standard deviation until the data is cleared from the window.

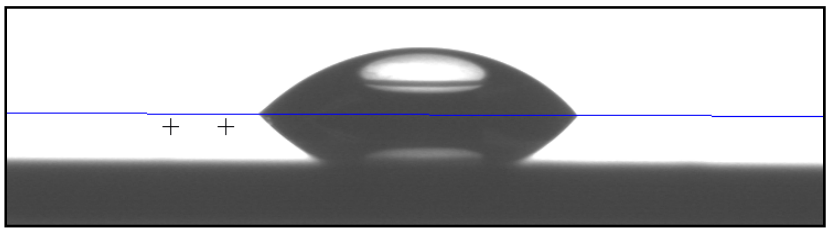


Figure A.4. Image a water drop with automatically corrected baseline and "+" for manual baseline correction.

A4. Variable-Angle Ellipsometry Procedure

Building a Model of the Surface

1. Open the Igor software from the desktop of the computer.
2. Open the Ellipsometry browser from the “bi” tab (Figure A.5).
3. Select the “Material” tab. Click Load to use previously created conditions. To create specific materials not found in the provided list, select “Cauchy” from the drop down list and click “New”. In the bottom right frame, put the appropriate refractive index value for the material. If the value is unknown, a good guess for an organic film is 1.5. In the left frame, the name of the material can be changed.
4. After all materials have been added, select the “Model” tab.
5. Click “New”. The name can be changed in the left frame. Check the “Model Edit Mode” box. Check the “Angle of Incidence” box and change the values to 35° for the minimum and 80° for the maximum.
6. To create the model, select the desired material in the left frame by a single click, and then double click the appropriate layer in the bottom right frame. Click “Insert Layer” and repeat until all layers of the surface have been assigned. In the bottom right frame, layer thicknesses can be entered, either from an initial guess value or from a previous experiment (as in a middle layer in which the thickness was determined in an earlier experiment).

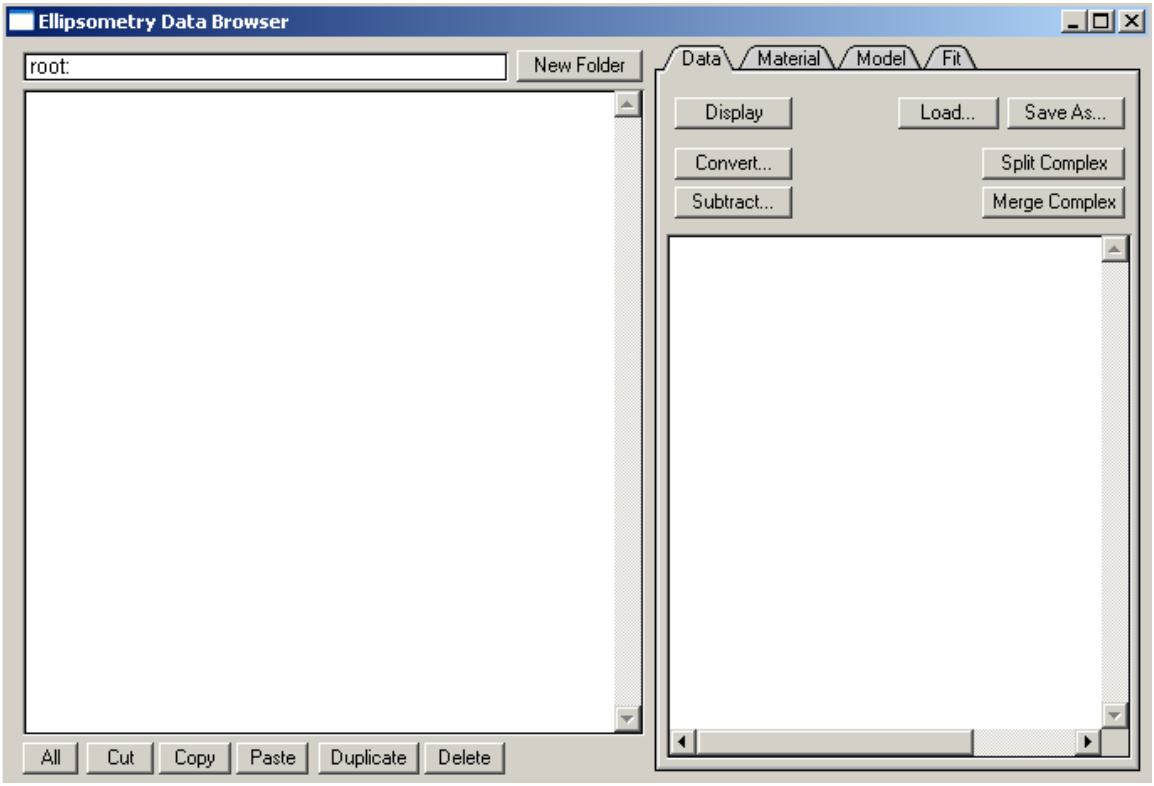


Figure A.5. Ellipsometry Browser window.

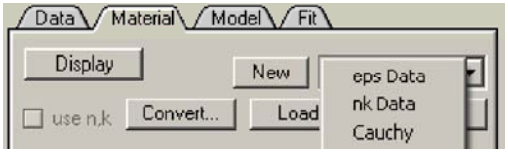


Figure A.6. Material tab of the Ellipsometry Browser.

Measuring the Thickness of a Surface

1. Open the Igor software from the desktop of the computer.
2. Open the PicoElli Control panel from the "bi" tab (Figure A.7).
3. Place sample on the stage and align the sample.
    - Check "Link" in the PicoElli Control Panel, type in 90° and click "Go".

- Put the pinhole on the left arm of the instrument.
- Change the angle to 80°, click “Go”, and check to see if the beam is aligned with the pinhole. If it is not, use the dials on the stage to adjust the sample position until the beam is aligned with the pinhole.
- Change the angle to 35°, click “Go”, and repeat the previous alignment adjustments until beam is aligned with the pinhole.
- Repeat this alignment until the beam is aligned at both angles.
- After alignment, bring arms to 90° and remove the pinhole. Return the arms to 80° when pinhole has been removed.

4. Click “Reset” in the PicoElli Control Panel. Check that that Polarizer and Analyzer are both at 45.
5. Check the “Variable Angle” box. Set the start angle to be 80°, step of -1, with 46 points and an interval of 2.
6. Click “Measure” to begin the experiment. Check the “Complex” box. In the name field, create an appropriate name for the run. When the measurement is complete, click “Make a Copy”.
7. From the “Fit” tab in the Ellipsometry browser (Figure A.8), check the “set model & target” box. In the left frame, select the model and file to be fitted by double-clicking. In the bottom right frame, check the box of the desired layer to be fitted and click “Execute Fit”.

8. The thickness will appear in the KO column of the pop-up window and the units are nanometers. (Note: Use a Microsoft Excel spreadsheet to save this number and to calculate averages and standard deviations.)

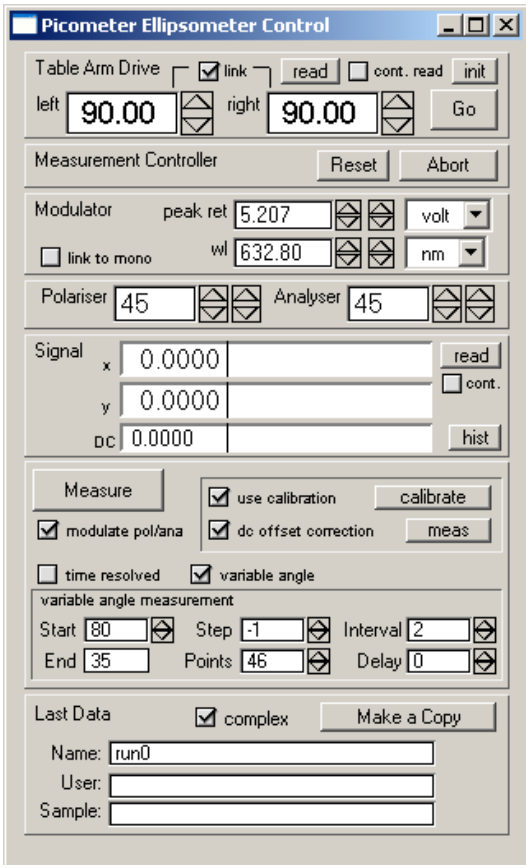


Figure A.7. PicoElli Ellipsometer Control panel.

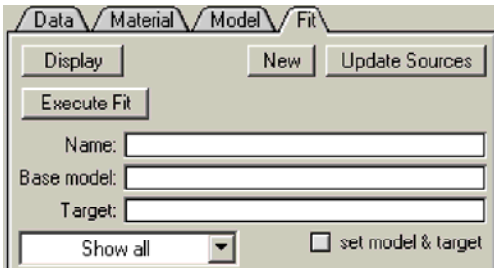


Figure A.8. Fit tab of the Ellipsometry Browser.

Appendix B

Determining Optimum Conditions

B1. Initial Tests

To find the optimal reactions conditions, initial tests were run to determine which solvents and weight percentages to use. Figure B.1 displays the initial test run of the materials to be used in the attachment procedure. These samples were all done on PGMA-modified surfaces (no ellipsometry was done). The sample ID "1" signifies that the PGMA was used in a 1.5 wt% and "2" means 3.0 wt % was used. Samples 1a, 1b, 1c, and 2a are 3-butyn-1-ol; samples 1d, 1e, and 2b are 1,6-diaminohexane; and samples 1f and 1g are 2-(aminomethyl)-2-methyl-1,3-propane diamine. All of these were used as a 1M solution in toluene. Samples 1a, 1d, 1g, 2a, and 2b were conducted at 65° C; samples 1c, 1e, and 1g were conducted at 50° C; and sample 1b was conducted at 80° C. The copolymer used is p(VP-co-VDMA) in a 1 wt% solution in THF.

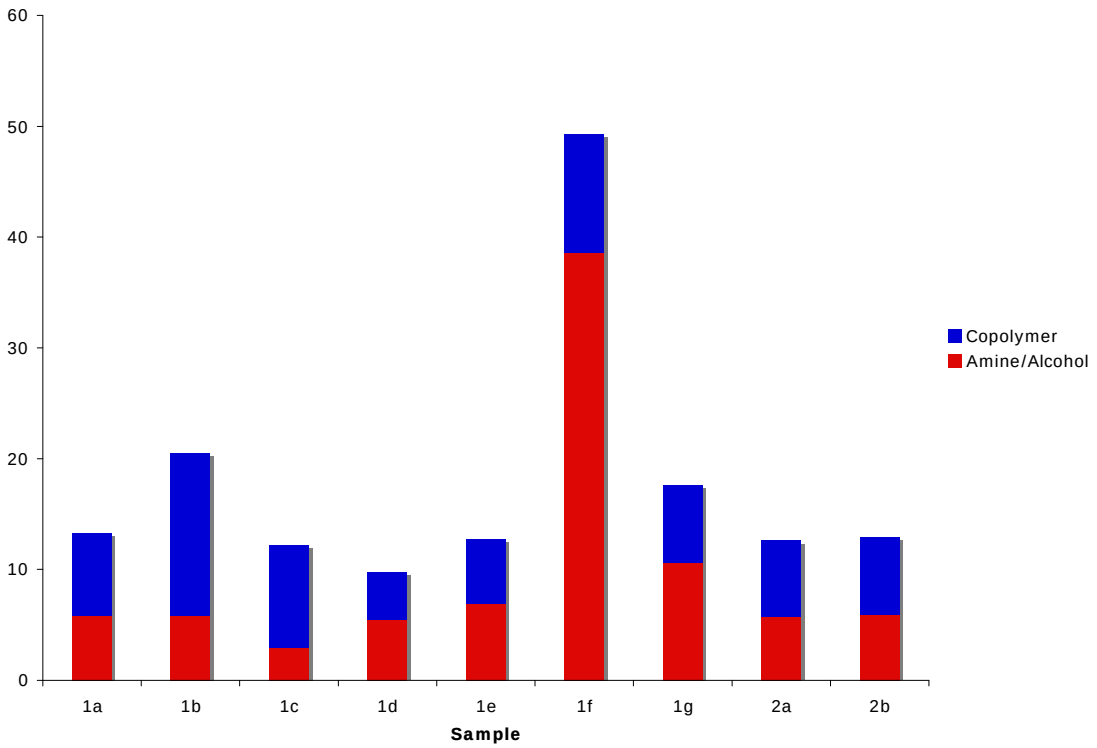


Figure B.1. Initial experiment with material to be used for attachment procedure.

B2. Amine Attachment

In order to best create a repeated procedure, the kinetics of the diamine attachment step was explored. Since the temperature chosen was 80° C, only time was manipulated in determining the procedure. The materials, concentrations, and general procedure used followed that found in Appendix A.1. Figure B.2 shows the results from this study. It is obvious that 3 hours is the optimal time for creating a thick uniform amine-functionalized layer.

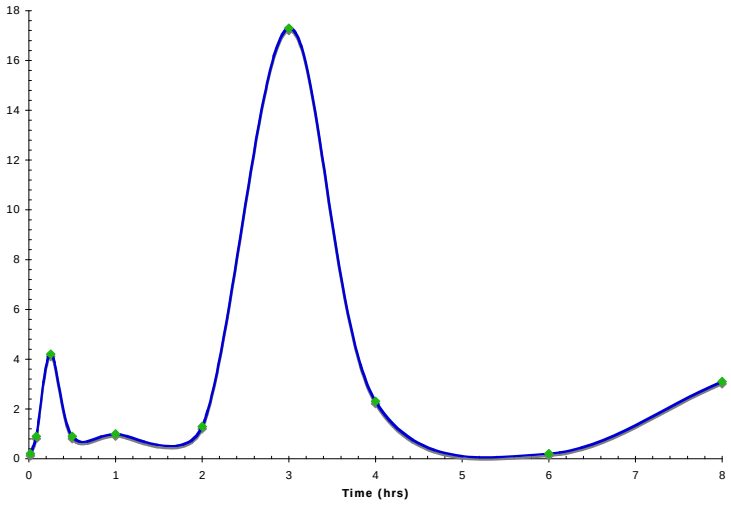


Figure B.2. Kinetics of Diamine attachment at 80° C.

B.3. Copolymer Attachment

The following figure shows the kinetics study run on the p(VP-co-VDMA) copolymer. The temperature of the reaction remained constant at room temperature, while the time was allowed to change. Another study was conducted in which the same experimental procedure was followed except that the reaction took place in a water bath at 30° C. In this study, no growth for the p(VP-co-VDMA) layer was measured.

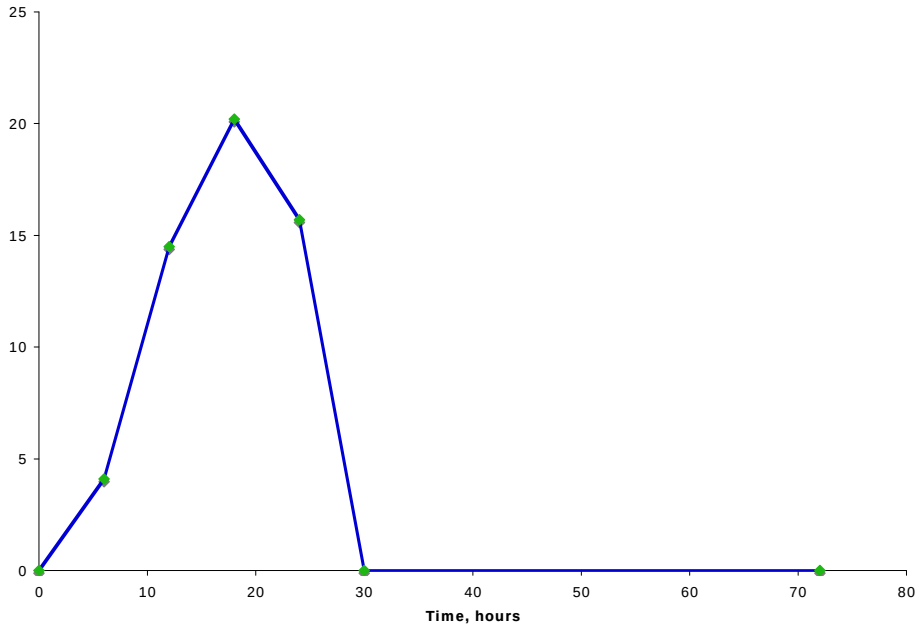


Figure B.3. Kinetics of copolymer attachment at room temperature.

Appendix C

Supplemental Data

C1. XPS Data

In an attempt to further support the IR results, the samples along each step of the construction process underwent XPS data measurements at Oak Ridge National Laboratory. The following scans aided in identifying the chemical changes that occurred during the attachment process. We found that the scans, along with other characterization methods, proved the presence of the anticipated chemical structures.

Silicon Oxide Thickness		
Sample	Ratio	Thickness / nm
JEB 1	Si2p Ox / Si2p elemental	0.83
JEB 2	Si2p Ox / Si2p elemental	0.82
JEB 3	Si2p Ox / Si2p elemental	2.1

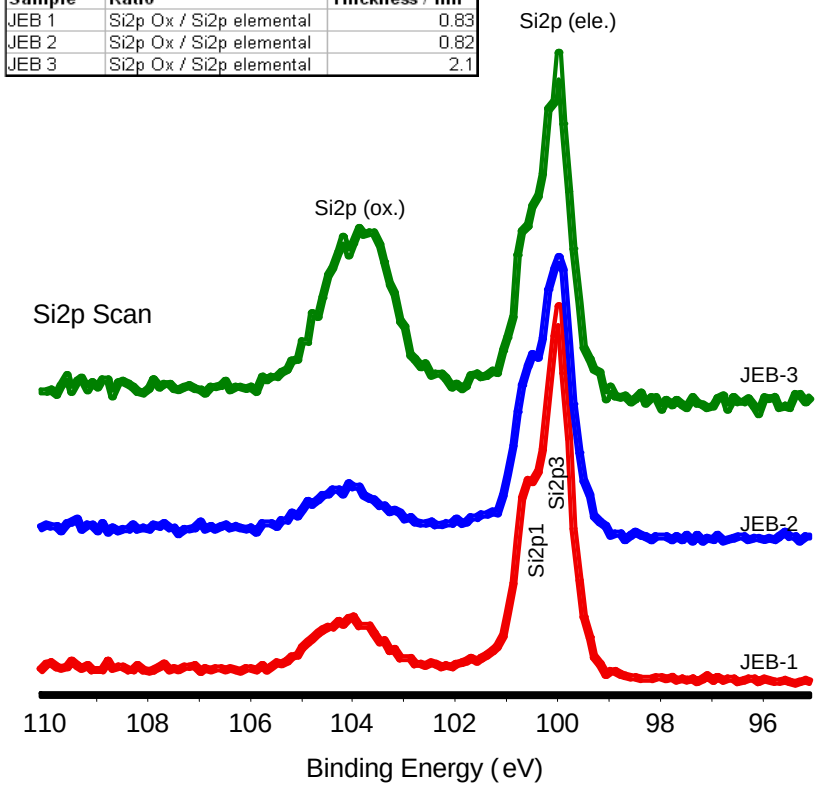


Figure C.1. Si signal from XPS on attachment procedure samples.

The figures below show the spectra and fits of the C 1s, O 1s, and N 1s core level in order to deduce the species contributing to the signal. The fits are seen as individual peaks in the spectra, and atomic composition resulting from the fits is shown in the corresponding tables.

JEB-1		
Element	**Peak BE**	**At. %**
C-C and C-H	284.8	26.6
C-N (amine)		0
C-O-C and C-O-H	286.5	24.8
C(=O)-N (amide)		0
O-C=O	288.7	8.7

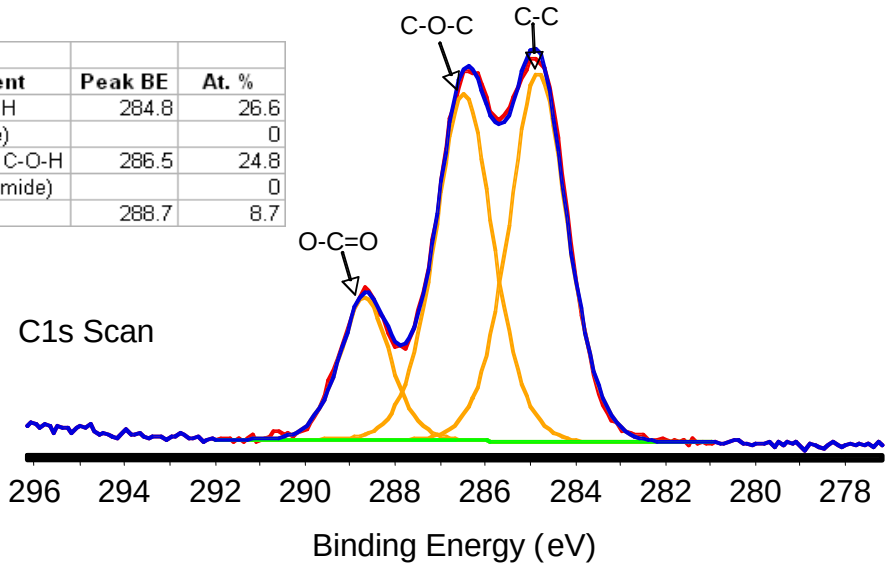


Figure C.2. C 1s scan of sample JEB-1 (PGMA on Si wafer).

JEB-1		
Element	**Peak BE**	**At. %**
O1s A	532.0	16.2
O1s B	533.2	12.5

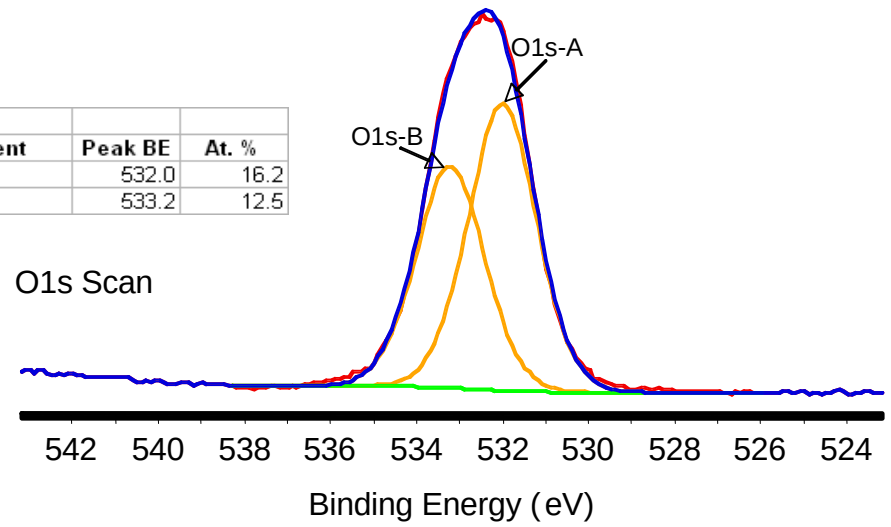


Figure C.3. O 1s scan of sample JEB-1 (PGMA on Si wafer).

JEB-2		
Element	**Peak BE**	**At. %**
C-C and C-H	284.9	38.3
C-N (amine)	286.0	5.5
C-O-C and C-O-H	286.5	11.4
C(=O)-N (amide)	287.7	3.0
O-C=O	288.7	6.5

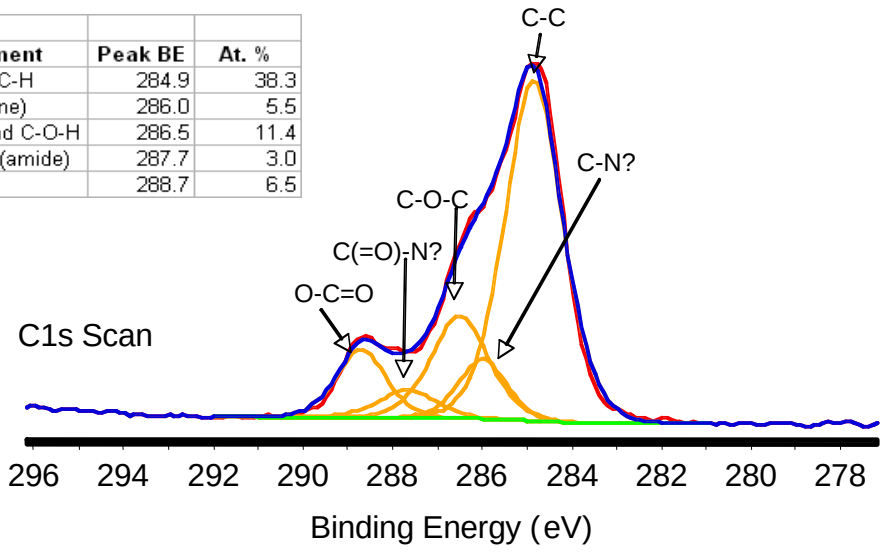


Figure C.4. C 1s scan of sample JEB-2 (PGMA with diamine attachment).

JEB-2		
Element	**Peak BE**	**At. %**
Si2p (ele.)	100.0	5.4
Si2p (ox.)	104.1	1.3

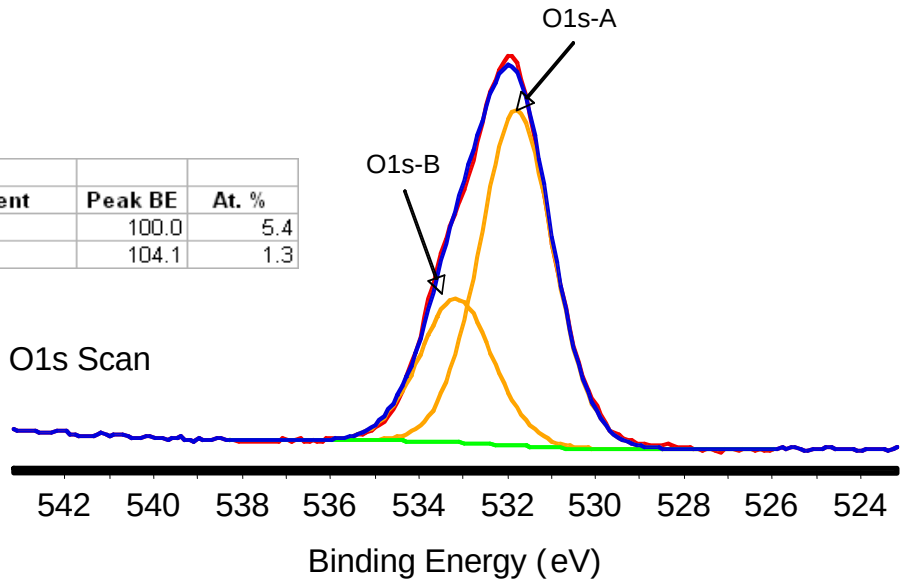


Figure C.5. O 1s scan of sample JEB-2 (PGMA with diamine attachment).

JEB-3		
Element	**Peak BE**	**At. %**
C-C and C-H	284.9	49.6
C-N (amine)	286.0	9.1
C-O-C and C-O-H	286.6	2.8
C(=O)-N (amide)	287.7	7.7
O-C=O	288.8	1.6

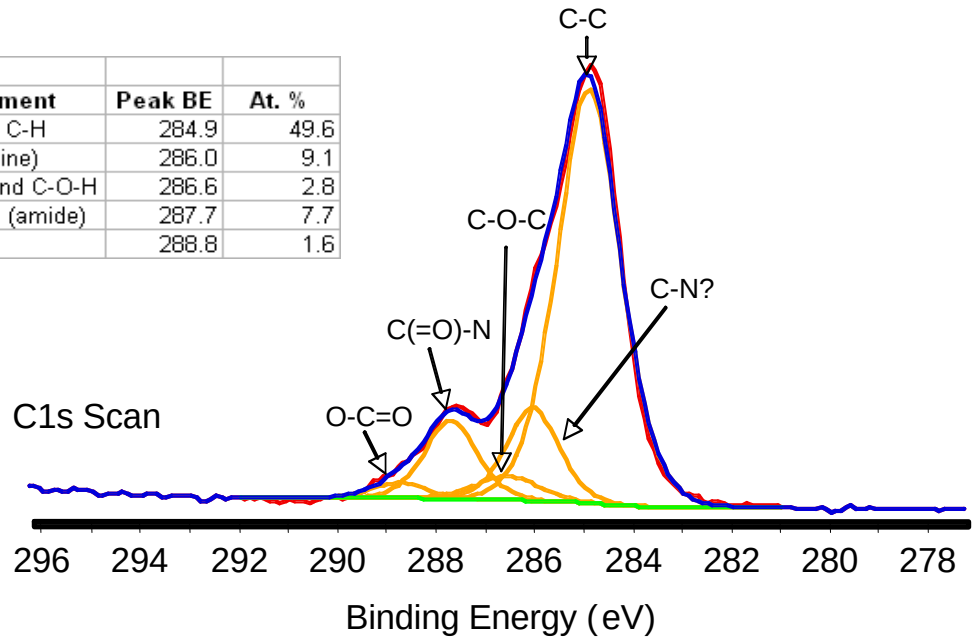


Figure C.6. C 1s scan of sample JEB-3 (Amine-modified surface with p(VP-co-VDMA) attachment).

JEB-3		
Element	**Peak BE**	**At. %**
O1s A	531.4	12.8
O1s B	533.0	2.9

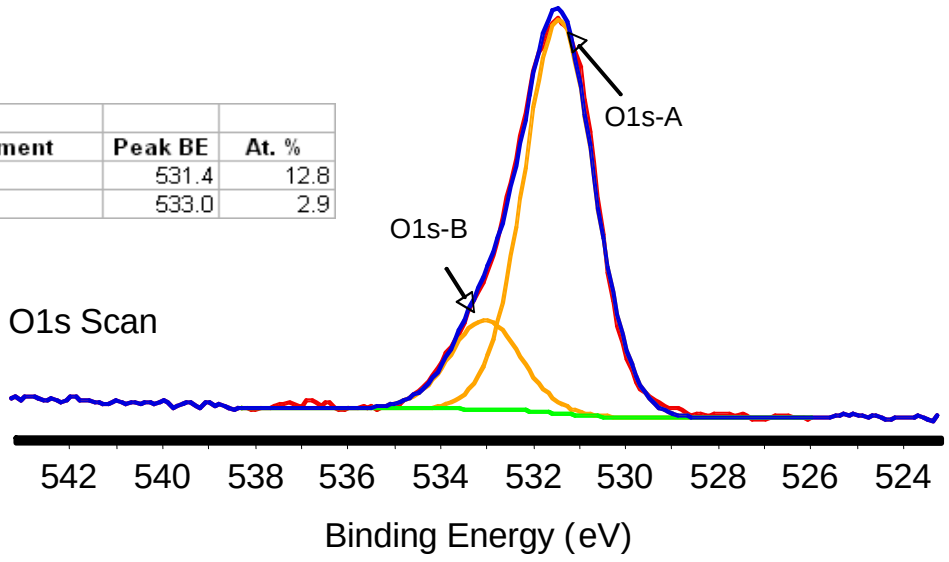


Figure C.7. O 1s scan of sample JEB-3 (Amine-modified surface with p(VP-co-VDMA) attachment).

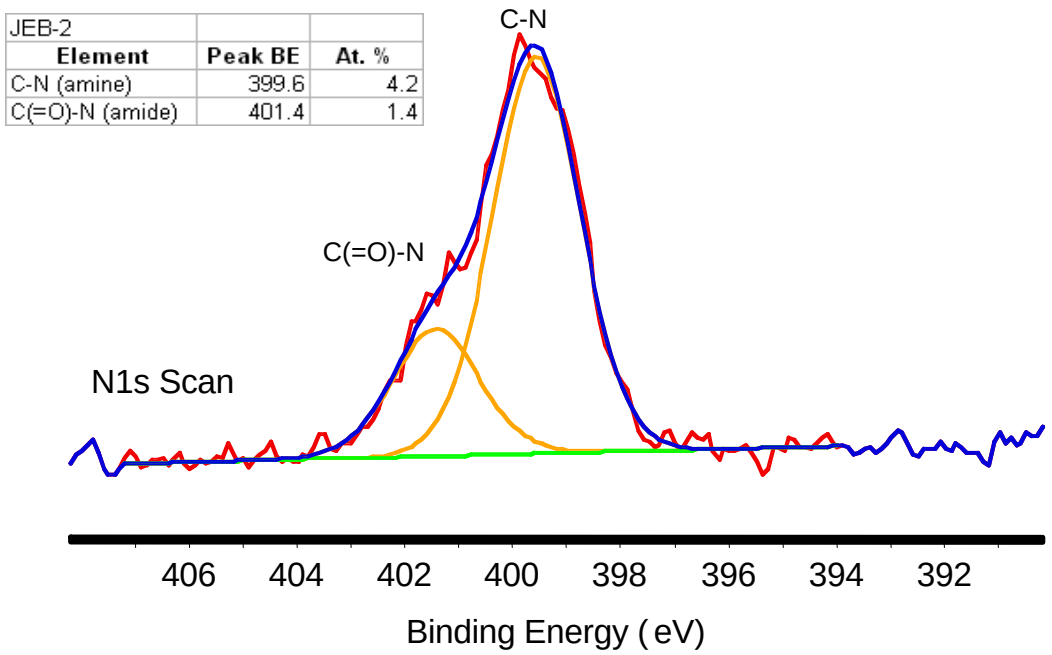


Figure C.8. N 1s scan of sample JEB-2 (PGMA with diamine attachment).

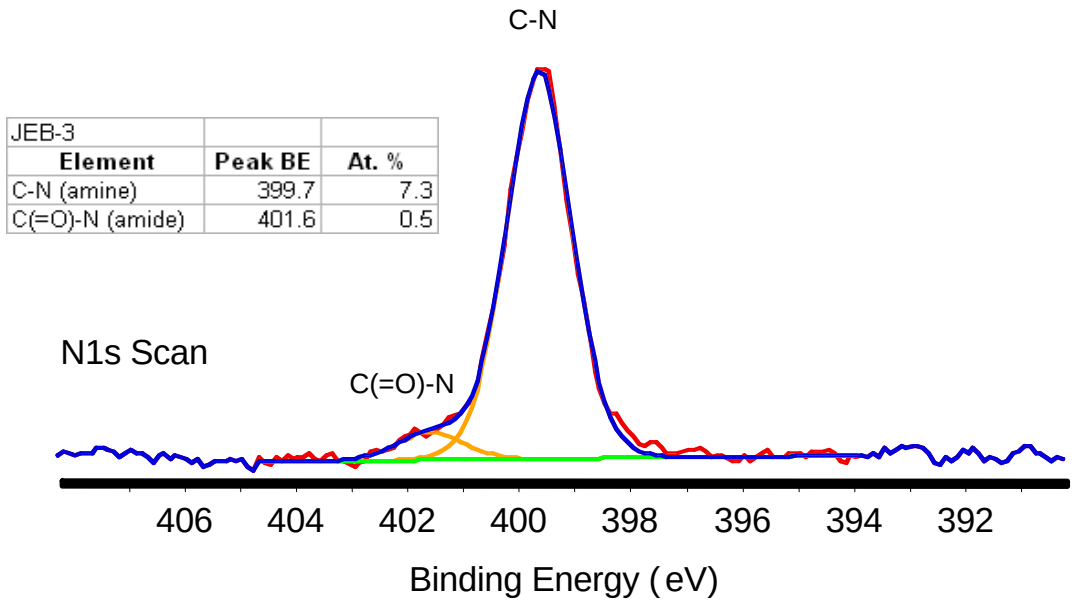


Figure C.9. N 1s scan of sample JEB-3 (Amine-modified surface with p(VP-co-VDMA) attachment).

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