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Nanoscale Polymeric Coatings that Inhibit Adsorption of Proteins

by

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ABSTRACT

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The ability of an artificial surface to resist the non-specific adsorption of proteins in biological solutions greatly improves its biocompatibility – a desirable property to overcome many problems across the biomedical and biochemical processing areas.

This thesis demonstrates the effectiveness of surface-initiated atom transfer radical polymerization for the synthesis of hydrophilic, protein resistant coatings with controlled thicknesses in the nanoscale regime (1-100 nm). The strategy uses the surface initiated atom transfer radical polymerization of commercially available oligo(ethylene glycol) methacrylate monomers with different chain lengths and either hydroxyl or methoxy group terminations.

This thesis evaluated the effects of chain length and end group of the monomers on the kinetics of their polymerization and the protein resistance of the resulting films. Protein adsorption for plasma proteins (lysozyme, fibrinogen, and albumin) as measured by XPS shows that, these coatings show remarkable resistance against protein adsorption that depends on the attributes of the monomer.
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Chapter 1

The non-specific adsorption of proteins

The non-specific adsorption of proteins onto surfaces is a major problem affecting advances in biotechnology and the development of biomedical devices. For example, the adsorption of plasma proteins onto cardiovascular implants causes thrombus development [1]. Similar effects are observed in the fouling of contact lenses by tear proteins [2].

Often, the adsorbed protein layer interacts with other biomolecules and cells, leading to their adsorption on surface. An example is the formation of a biofilms to yield a colony of immobilized bacteria on a surface that then show enhanced resistance to antibiotics due to their robust structure. Generally, an adsorbed protein layer is necessary for the growth of such biofilms [3].

Protein adsorption can limit the effectiveness of surfaces that provide the active component for sensors. Biosensors are a subclass of sensors that are used for detection of antibodies, antigens and other biomolecules. A typical biosensor consists of probe molecules immobilized on a substrate. The probe molecules generate a signal upon binding with the target molecules. The non-specific adsorption of proteins on these substrates lowers the signal from these sensors and often causes higher background (noise) levels [4].

The inhibition of protein adsorption is even more critical for applications involving substrates with nano or micron sized features such as nanoparticles, microchannels and membranes due to the large surface area that they present for interactions. Also, in these applications, the thickness of the adsorbed layer is often
comparable to the particle/pore size. Hence, there is a need across many applications to develop nanoscale coatings that inhibit the non-specific adsorption of proteins.

The development of these "antifouling" coatings forms an active area of research within a broader field of biointerface science – the science of controlling biomolecular interactions at interfaces[5]. This area also has implications for the development of new biomaterials because protein adsorption is often the first step in a cascade of adsorption events that occurs when a foreign object is placed in contact with blood or other biological media. As such, the inhibition of protein adsorption to a surface also improves the biocompatibility of a material.

§ 1.1 Protein structure:

Proteins are linear copolymers of twenty different L-amino acids. Each of these twenty basic building blocks is characterized by a different size, shape, charge, hydrophobicity and reactivity. Because of the presence of these functional groups, proteins are capable of interacting with surfaces through various forces such as long-range electrostatic interactions, hydrophobic interactions, and hydrogen bonding.

Protein molecules are formed by linking several amino acids together by amide linkages (-NHCO-). While the primary structure of a protein is that of a chain of monomers, the interactions among various amino acid residues through hydrogen bonding and hydrophobic interactions as well as disulfide linkages result in the folding of the polypeptide chain in a characteristic 3D structure, which is the native conformation of the protein.

In water-soluble proteins, the polypeptide chain is compactly folded to give a roughly spherical structure with most of the hydrophilic amino acids on the outside.
These amino acids are exposed to water and most of the hydrophobic amino acids located inside the sphere. Proteins having this type of structure are known as ‘globular proteins’. Other shapes of protein molecules are possible, but these are not water-soluble. Since we are concerned with the adsorption of proteins from aqueous solutions, the following discussion focuses on globular proteins. Globular proteins form the largest group of functional proteins (e.g. enzymes, immunological proteins, and transport proteins). For a majority of practical applications, the adsorption of globular proteins is the most relevant [6].

Globular proteins are low entropy structures that are stabilized by various interactions among the amino residues. Among these, hydrophobic interactions play a major role in stabilizing the globular structure. The globular structure is only marginally stable (typically a few tens of kJ per mole of protein[7]) and even mild changes in the environment (Temperature, pH, ionic strength) can cause unfolding of the protein.

§ 1.2 Factors that affect protein adsorption: [8]

At a constant temperature and pressure, adsorption proceeds spontaneously if the Gibbs energy of the system decreases (eq 1).

\[ \Delta G_{ads} = \Delta H_{ads} - T \Delta S_{ads} < 0 \quad \text{---------}(1) \]

Entropic effects that drive adsorption are the unfolding of a protein at an interface and the release of counterions and water molecules from, the surface and the protein during the adsorption process. Enthalpic contributions are the various attractive interactions between the protein and surface, mainly, electrostatic, hydrophobic, and van der Waals interactions.
The chemical composition of substrate surface determines the nature of its interactions with a protein and other chemical species present in the vicinity that could adsorb to a surface. For example, metal oxide surfaces adsorb proteins mainly through the electrostatic interactions with proteins. Polymeric materials can interact with proteins through various polar and nonpolar interactions with various functional groups (–COOH, NH₂, aromatics etc), presented by the material to the aqueous medium.

The physical properties of the surface also affect protein adsorption. Experimentally, it has been observed that hydrophobic surfaces tend to adsorb more proteins than do hydrophilic surfaces [9]. Even Teflon®, considered a “non-stick” surface, rapidly adsorbs proteins from blood plasma on account of its hydrophobic nature. Recent AFM measurements of protein surface interactions using self-assembled monolayers (SAM) as model surfaces also indicate that the adhesional strength is more in water for SAMs that express hydrophobic functional groups [10, 11].

This observation can be explained by the minimum interfacial free energy hypothesis postulated by J.D. Andrade [12]. According to this hypothesis, the driving force for adsorption is a lowering of the surface free energy in an aqueous solution, resulting in a more “water-like” interface. Hydrophilic surfaces have lower interface free energies in aqueous solutions than do hydrophobic surfaces. Therefore, the driving force for adsorption is lower on hydrophilic surfaces and such systems are generally targeted for producing surfaces with non-fouling characteristics.

Some general correlations have been developed between protein properties and their affinities for surfaces. Larger proteins are more likely to interact with the surface because they can contact the surface at more sites. Hence, adsorption is more for bigger
proteins. Protein adsorption is greatly influenced by charge and its distribution on the surface of the protein molecule. Adsorption also depends on the stability of a protein structure because an unfolding of a protein can occur upon adsorption. The unfolding of a protein increases the conformational freedom of the peptide chain and also makes more sites available for protein-surface contacts. Therefore, proteins with “soft” structures (i.e. less stable due to weaker intramolecular cross linking) are more likely to unfold at interfaces.

§ 1.3 Properties of the proteins used in this study:

In this thesis, I evaluated the ability of polymeric coatings to inhibit the adsorption of three different proteins having different sizes and isoelectric points. Table 1 lists the properties of these proteins.

Table 1: Properties of lysozyme, albumin, and fibrinogen

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Wt (Da)</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>14,307</td>
<td>11.4</td>
</tr>
<tr>
<td>Albumin</td>
<td>66,200</td>
<td>5.1</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>340,000</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Fibrinogen, a large protein (450 x 90 x 90 Å³), is composed of three globular units connected by a helical coil. It is the “stickiest” among all plasma proteins and plays a crucial role in wound healing, clot formation, and platelet adhesion. Lysozyme is a small protein (46 x 30 x 30 Å³) and it is much less prone to unfolding at interfaces. It also provides a positively charged protein to contrast with the other examined species. Albumin comprises about 60% of the blood-plasma proteins and it exhibits a surface passivating behavior that results from its nonspecific adsorption properties.
Chapter 2

Polymer brushes of poly (ethylene glycol): Antifouling properties

Poly (ethylene glycol), often abbreviated as PEG, is one of the most widely used polymers for generation of biocompatible materials. It has a simple chemical structure:

\[-\text{CH}_2-\text{CH}_2-\text{O}]_n-\]

PEG is a water soluble, nontoxic, and non-immunogenic polymer [13].

PEG has some peculiar physical properties. For example, PEG chains prefer to adopt a gauche state about its constituent OC-CO bonds, which makes them highly flexible [14]. PEG chains can form hydrogen bonds with water molecules. It has been determined from NMR relaxation time measurements that, there is one water molecule bound per repeat EG unit in an aqueous solution [15].

In the early 80s, it was discovered that surfaces with grafted PEG chains exhibit reduced protein adsorption [16]. Since then, there have been many theoretical and experimental investigations into the mechanism for the inertness of PEG [17-31].

Jeon and Andrade [17] proposed a simple model based on de Genne’s theory of polymer brushes [18, 19]. This model explains the inertness of PEG chains in terms of the steric repulsion generated by the protein as it approaches the surface through a highly solvated PEG layer. Steric repulsion is caused by dehydration and confinement of the highly flexible, hydrated PEG chains. Accordingly, a high surface density and a long chain length of PEG is necessary for optimal protein resistance, with the density being
more important than the chain length. A limitation of the Jeon-Andrade model is that it is valid only for very long chains (n > 200) in the brush regime.

Whitesides et al. [20] experimentally demonstrated that longer chain lengths may not be required for protein resistance. Using thiolate based self-assembled monolayers on gold, they determined the correlation between chain length, surface density, and protein resistance for short EG chain lengths n = 1 to 17. Their results indicate that longer chains were resistant at lower mole fractions in the monolayer. The minimum mole fraction of chains that prevent adsorption was found to be proportional to n⁻⁰.⁴, where n = EG chain length.

Szleifer et al. [21-23] proposed a modified theory using a generalized single-chain mean-field theory approach and applied it for studying adsorption of lysozyme on PEG-grafted surfaces. They calculated the potentials of mean force of the protein with the surface as a function of grafting density and amount of adsorbed protein. This approach was also applicable to systems containing small chain lengths (n > 6) and predicted that the surface grafting density of the polymer is the most important parameter while the chain length has a weak effect.

Grunze et al. [24, 25] found that chain conformations and their interactions with water are the determinants of inertness of OEG-containing self-assembled monolayers. Their theoretical and experimental work suggests that crystalline helical and the amorphous forms of the OEG chains are resistant to proteins while densely packed “all-trans” forms are not protein resistant.

Overall, the mechanism for the inertness is still not clear despite all these investigations. However, several researchers have experimentally demonstrated the
ability of PEG to prevent protein adsorption. Previously, PEG-grafted surfaces have been prepared by physical adsorption [26], covalent coupling [27], glow discharge polymerization [28], UV-induced graft polymerization of PEG methacrylate macromonomer [29], chemical vapor deposition of ethylene oxide [30], and PEG coupled self-assembled monolayers [31]. However, these techniques have several limitations regarding control of thickness, grafting density, uniformity, and stability.
Chapter 3

Atom Transfer Radical Polymerization (ATRP):

Fundamentals and applications for surface-initiated polymerization

Free radical polymerization is one of the most useful methods for polymer synthesis, partly because it can be applied to a large number of monomers and requires relatively moderate conditions. It is estimated that 50% of the synthetic polymers are prepared by free radical polymerization [32]. However, one of the major problems of conventional free radical polymerization is the lack of control over the structure of polymer molecules; it is not possible to obtain polymers with precisely defined end groups and narrow polydispersities.

The kinetics of free radical polymerization involves the following elementary steps:

Initiation: \[ I \xrightarrow{k_i} I^* \]

\[ I^* + M \xrightarrow{k_i} P_1^* \] \hspace{1cm} \text{(1)}

Propagation:

\[ P_1^* + nM \xrightarrow{k_p} P_n^* \] \hspace{1cm} \text{(3)}

Termination:

\[ P_n^* + P_m^* \xrightarrow{k_t} P_{(n+m)}^* \] \hspace{1cm} \text{(4)}

Chain transfer:

\[ P_n^* + SX \xrightarrow{k_{tr}} P_n + SX^* \] \hspace{1cm} \text{(5)}

Where, \( I \) = Initiator, \( M \) = Monomer, \( P_n \) = Polymer, and \( SX \) = Solvent.

* indicates activated form (radical) of the corresponding species.
Polymer chains grow by the addition of monomer units to an active end (eq 3). While the addition of the monomers maintains active sites for further polymer growth, chain breaking events (such as chain transfer and termination reactions) lead to a loss of active functionality. Since there are a number of possible mechanisms for chain breaking (only one is shown above for simplicity), there are various possibilities for the polymer end group, resulting in a distribution of end groups. Also, the molecular weight distribution for the polymer is broad due to the occurrence of random termination and transfer events.

A living polymerization results if chain transfer and chain termination events are avoided by manipulation of reaction conditions [33]. In a typical living polymerization process, polymer chains continue to grow until the monomer is completely consumed so that further addition of monomers results in polymer growth. As a result, living polymerization systems have precisely controlled end functionalities. Additionally, if the rate of initiation is fast and there is a rapid exchange between species of various reactivities and lifetimes, the molecular weight distribution is narrow [34]. Because all the chains grow at the same rate, there is a linear increase in molecular weight with time. It is also possible to control the structure of the polymer chains (block copolymer, star polymer etc.) through living polymerization.

Indeed, these advantages have been demonstrated in the cases of living cationic [35], anionic [36] polymerizations [37] and also living ring opening metathesis polymerization [38]. Living free radical polymerizations are, however, not possible because termination reactions between growing radicals are unavoidable as unlike cations or anions, free radicals can combine each other. Therefore, the only way to control free
radical polymerization processes is use an approach that minimizes the contributions of side reactions (termination etc.) by keeping the concentrations of growing radicals low. This can be achieved by introducing a capping agent that reacts reversibly with the growing radicals to generate a non-radical species and thereby limits the concentration of active radicals (Figure 3.1). This type of polymerization is known as "pseudo-living" or controlled/living radical polymerization as it mimics all the features of a typical living polymerization.

\[
\begin{align*}
P_n & \xrightarrow{K_{\text{act}}} X + Y \\
\text{K}_{\text{deact}} & \xrightarrow{K_p} X - Y + P^* \\
+ M & \xrightarrow{K_p} P_n
\end{align*}
\]

Figure 3.1: Capping agent (X-Y) establishes dynamic equilibrium between growing free radicals and dormant species.

Over the last decade this field has developed rapidly as evident by the publication of over ~ 4000 papers and the issuing of more than 400 patents on controlled radical polymerization [39]. Many strategies have been developed; few notable mechanisms are:

- **NMP** - Nitroxide mediated polymerization [40]
- **ATRP** - Atom transfer radical polymerization [41]
- **RAFT** - Reversible addition fragmentation chain transfer [42]

ATRP is by far the most widely applied strategy (~ 3000 papers and ~ 300 patents since the first report in 1995 [43]). ATRP utilizes a transition metal catalyst (typically a copper complex with various bidentate ligands) for reversible deactivation of growing
free radicals. Figure 3.2 shows the generally accepted mechanism of copper catalyzed ATRP.

Initiation:

\[
\begin{align*}
R-X + \text{Cu(I)/(bpy)}_2 & \xrightarrow{K_a} R^* + \text{X-Cu(II)/(bpy)}_2 \\
& \xrightarrow{K_d} R-M X + \text{Cu(I)/(bpy)}_2 & \xrightarrow{K_d} R-M^* + \text{X-Cu(II)/(bpy)}_2
\end{align*}
\]

Propagation:

\[
\begin{align*}
P_nX + \text{Cu(I)/(bpy)}_2 & \xrightarrow{K_{act}} X-\text{Cu(II)/(bpy)}_2 + P_n^* \\
& \xrightarrow{K_{deact}} P_n
\end{align*}
\]

Figure 3.2: Mechanism of ATRP

The following criteria are satisfied in ATRP by choosing a proper initiator and catalyst:

\[
[R-X] : [Cu(I)] : [M] = 1 : 1 : 1000
\]

\[
\begin{align*}
K_a^o & \ll K_d^o & K_{act} & \sim 1 \text{ M}^{-1} \text{s}^{-1} \\
K_a^o & \gg K_{act} & K_{deact} & \sim 10^7 \text{ M}^{-1} \text{s}^{-1} \\
K_{deact}[X-\text{Cu(II)/(bpy)}_2] & >> K_p[M] & K_p & \sim 10^3 \text{ M}^{-1} \text{s}^{-1}
\end{align*}
\]

ATRP initiators (R−X) are molecules that contain an activated halogen atom (X). The employed transition metal catalyst is capable of shuttling between two oxidation states (here Cu(I) and Cu(II)) by the transfer of a halogen atom. Similar to conventional
polymerization, radicals in ATRP participate in initiation, propagation and termination reactions. However, the formation of radicals during the ATRP process is reversible. Hence, their steady state concentration is low because of persistent radical effect \([44]\) (i.e. the equilibrium between the activation \((k_{\text{act}})\) and deactivation \((k_{\text{deac}})\) processes is shifted to the left-hand side). This decrease in concentration of radicals reduces the termination reactions because propagation is a first-order reaction and termination is second-order w.r.t. radicals. As a result, polymers with predictable molecular weights, narrow molecular weight distributions, and end functionalities can be synthesized.

Typical polydispersities observed in ATRP are in the range of 1.2-1.3, which demonstrate its controlled nature. In contrast, the polydispersities in most conventional polymerization reactions are 1.5 -2.0. ATRP also provides control over end group functionality of the polymer. During the polymerization, most of the chains (>95\%) are present in a deactivated form \((P_n-X\) where, \(P_n = \) polymer chain). Hence, after quenching the polymerization, chains terminated with a halogen atom are obtained. These chains can then be used for subsequent polymerization of another monomer and for synthesis of diblock copolymers \([45]\).

As a free radical polymerization method, ATRP is tolerant to a variety of functional groups. Therefore, it can be used to incorporate a variety of functionalized acrylate, methacrylate, styrene, and acrylonitrile monomers into a growing polymer chain without any protection-deprotection chemistry. In terms of operating conditions, its only drawback is its oxygen sensitivity due to radical propagation mechanism employed for polymer growth.
An advantage of ATRP over other living polymerization methods is that it can be performed in water and other protic solvents, whereas most ionic methods require anhydrous conditions. ATRP proceeds at faster rate in water or in polar solvents than in non-aqueous media. The addition of sufficiently large amounts of the deactivating Cu(II) complex to the solution ensures that a control over the polymerization reaction is retained. [46-54]

§ 3.2 Polymer brush:

Polymer brushes refer to an assembly of polymer chains that are tethered by one end to a surface or an interface. When the grafting density is high enough so that the distance D between adjacent chains is lower than twice the radius of gyration (Rg) of the free polymer chain, the chains are forced to adopt stretched conformations. (Figure 3.3) At lower grafting densities, the chains are present in collapsed form. (D > Rg)

![Diagram](image)

**Figure 3.3:** Effect of grafting density on conformations of the polymer chains. When the separation distance (D) is less than the radius of gyration (Rg) for the polymer, the chains adopt a "brush" structure.
The formation of stable polymer brush requires a robust method of surface modification. In this project, polymer brush formation was used to generate a surface with hydrophilic properties and make it protein resistant.

_Synthesis of Polymer Brush_: Robust polymer brushes can be formed by covalently attaching polymer chains to a surface. Two commonly employed methods for their formation are described bellow.

**A)** The "_grafting to_" approach: Here a polymer brush is formed by reacting preformed end-functionalized polymer molecules to functionalities on a surface (Figure 3.4 a). In this case, only a small amount of polymer can be immobilized onto the surface because the macromolecular chains have to diffuse through existing polymer film to reach the reactive sites on the surface. These steric barriers become more pronounced as the tethered polymer film thickness increases.

**B)** The "_grafting from_" approach: This strategy can be achieved using a surface-initiated polymerization process (Figure 3.4 b). First, the substrate of choice (planar or particle) is modified with an initiator-bearing self-assembled monolayer. These monolayers can be formed on almost any surface, as long as the anchoring functionality is appropriately selected. For example: thiols on gold, silanes on glass, Si/SiO$_2$ and plasma oxidized polymers provide convenient anchoring combinations. The initiator surface is then exposed to a solution containing a catalyst and the monomer (plus solvent if necessary). Generally, it is preferred that the polymerization is not only surface-initiated but also surface-confined, meaning that no polymerization occurs in solution. The "_grafting from_" method is generally more effective than the "_grafting to_" approach as the small monomer molecules can diffuse more readily to the reactive groups present...
at the exposed surface rather than to the underlying substrate. Thus, the polymer brushes obtained by this method have higher grafting densities (up to 85 mg/m²) as compared to those obtained by the “grafting to” method (typically 10-40 mg/m²).

Figure 3.4: Examples of tethered polymer chains formed by a) “Grafting from” and b) “Grafting to” methods. In a), monomers attach to a growing polymer chain. In b), a preformed polymer chain is directly attached to the surface.

§ 3.3 Surface-initiated ATRP:

One of the recent application of ATRP is in the field of surface-initiated polymerization where ATRP is used in the “grafting from” approach.[55-57]. It follows from the mechanism of ATRP that a majority of the polymer chains prepared using the initiator R-X have a well-defined structure as shown in Figure 3.5 a. In a surface-initiated ATRP process, the initiator is first immobilized onto a surface through the R group
Figure 3.5 b. Various strategies are available for this immobilization depending on the type of substrate (e.g. trichlorosilane SAM on silicon, thiol SAMs on gold, etc). ATRP is then initiated by this surface-immobilized initiator, the resulting polymer chains are generated as chemically attached material to the substrate surface. Analogous to controlling the degree of polymerization in a solution-phase reaction, the thickness of the grafted polymer film can be controlled in a surface ATRP reaction. (Figure 3.5)

a)

\[ R-X \xrightleftharpoons{\text{ATRP}} \rightarrow R-Y \]

Degree of Polymerization

b)

\[ \text{Surface} \xrightarrow{\text{ATRP}} \text{Polymer} \]

Thickness

Figure 3.5: Examples of ATRP a) ATRP in solution and b) from a surface.
A challenge for surface-initiated polymerizations is that the polymer chains grow in close proximity and the termination probability is considerably higher than in the solution phase. For surface-initiated ATRP, the concentration of surface immobilized initiator (just a monolayer) is not sufficient to provide enough deactivating species to limit the number of free radical sites. Therefore, to keep these levels low, it is necessary to add either a sacrificial initiator [58, 59] or the deactivator in sufficient concentration to the solution. It is also important to dilute the catalyst concentration [60, 61].

Surface-initiated ATRP was first reported by Ejaz et al. [62] in 1998. Since then, many research groups have explored this technique for the synthesis of various functional polymeric coatings. Surface-initiated ATRP has been successfully applied to a variety of monomers (styrene [56], acrylates [56], methacrylates [63], acrylamides [64]), as well as on various flat (gold [65], silicon [66]), and curved substrates (nanoparticles [67, 68], carbon nanotubes (MWNT [69]; SWNT [70]), dendrimers [71]).

Surface-initiated ATRP, when performed in aqueous solutions, is very effective and can be used for synthesis of polymer films with thicknesses on the order of 100 nm. Bruening et al. have obtained poly (HEMA) films of up to 700 nm by surface-initiated ATRP in water [72].

ATRP has been applied towards the synthesis of surfaces with various tailored functionalities. An interesting feature of ATRP is that block copolymer brushes can be successfully prepared by using the end groups of a previously prepared film as initiators. In this manner, Bruening and Baker have prepared triblock copolymer brushes PMMA-b-PMA-b-PHEMA on gold surfaces [73]. Surface-initiated ATRP in combination with
various patterning techniques (microcontact printing [74], for example) has been applied to create patterned polymer brushes.
Chapter 4

Research Introduction

Research was directed toward the synthesis of grafted poly (oligo (ethylene glycol) methacrylate) films, that exposed PEG chains in high density by a surface-initiated ATRP reaction. (Figure 4.1)

![Diagram of grafted films with PEG chains]

Figure 4.1: Schematic representation of films containing PEG chains grafted to a polyacrylate backbone.

My strategy involved a two-step synthesis of the polymer brush shown in Scheme 1. A self-assembled monolayer (SAM) was formed by dipping plasma cleaned silicon wafers in 1 mM solution of initiator 1. This process created a surface coated with uniformly distributed molecules expressing terminal Br atoms as needed for the next step. Surface-initiated ATRP was performed from these Br-terminated SAMs in the next step. Surface ATRP with methacrylate monomer in presence of the copper catalyst gave polymer chains that were chemically attached to the surface (Figure 4.2).
Figure 4.2: Two step synthesis of poly (OEGMA) grafted films.

a) self-assembly of ATRP initiator 1
b) surface-initiated ATRP
For the targeted films, a variety of methacrylate monomers are available with different polyethylene glycol (EG) chain lengths (n) and either hydroxyl of methoxy terminal groups. (Figure 4.3)

![Chemical structures](image)

**Figure 4.3:** Commercially available methacrylate monomers as esters of oligo (ethylene glycol) with various chain lengths and end functionalities.

As differences in the molecular structure of the side EG chains could affect the ability of monomers to undergo polymerization and also the anti-fouling ability of the resulting polymer films, I evaluated monomers with chain lengths \( n = 4, 9, 23 \) (all methoxy capped) and \( n = 1, 5, 10 \) (Hydroxyl capped) in order to find the monomer with the most useful properties.

In a recent communication, Chilkoti et al. [75] have reported the effectiveness of such coatings prepared using methoxy capped monomer with \( n = 9 \). They have produced polymer films of \( \sim 15 \) nm in thickness and demonstrated the "anti-fouling" abilities of these films against fibronectin solution as well as against 100 % fetal bovine serum. In many applications, (e.g. a surface-based biosensor) thinner films may be required. Therefore, as part of my research I investigated the effect of film thickness on the inertness of these coatings to determine the minimum thickness required for their performance.
Chapter 5
Experimental Section

5.1 Materials:

Oligo (ethylene glycol) methyl ether methacrylate \((n = 4, 9 \text{ and } 23)\) and oligo (ethylene glycol) methacrylate \((n = 1, 5, 10)\) were obtained from Sigma Aldrich Co. The monomers were used as received and stored at 4 °C when not in use. Silane coupled initiator [1] was synthesized as per reported procedure [56]. Silicon wafers (100) were purchased from Si-Tech Inc. The wafers were diced into small pieces \((1 \text{ cm x } 1.5 \text{ cm})\). Water (Purity 18.2 MΩ-cm) was obtained from Barnstead Nanopure Diamond™ water purification system. CuBr (98 %) and CuBr₂ (99 %) were obtained from Sigma Aldrich Co. Bipyridyl (99 %) was obtained from Alfa Aesar Co. Fibrinogen (Source: Cat Plasma) was obtained from Sigma Aldrich Co.

5.2 Methods:

5.2.1 Experimental methods

Wafers were cleaned by treatment with piranha solution \((3:1 \text{ mixture of } \text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2)\) at room temperature for 10 minutes. After removal from piranha solution, the wafers were washed with plenty of millipore water and dried under nitrogen.

SAM formation was achieved by dipping the piranha cleaned samples in 4mM solution of the initiator in anhydrous toluene for 24 h. After removal from the solution, samples were washed with toluene and dried under nitrogen.
Surface-initiated polymerization was carried out under nitrogen atmosphere in a glove box to avoid exposure of the reaction mixture to oxygen. Following common procedure was followed for all monomers keeping monomer concentration constant at 0.5 M. Millipore water and methanol were deaerated by bubbling nitrogen through for 10 min. CuBr 195 mg, CuBr$_2$ 103 mg and bipyridyl 568 mg were dissolved in a solution containing 10 mL each of deaerated water and methanol to make a deep brown colored catalyst solution containing 68 mM CuBr, 23 mM CuBr$_2$ and 182 mM bipyridyl. This was followed by addition of requisite amount of monomer to make the solution 0.5 M w.r.t. the monomer. A two-neck round bottom flask was attached to shlenk line and degassed by two cycles of evacuation and backfill. (Catalyst + Polymer) solution was cannulated into the flask and the mixture stirred. This polymerization solution was further degassed by three “freeze and thaw” cycles and transferred into the glovebox. Polymerization was initiated by transferring the polymerization mixture into a vial containing initiator SAM. After required time interval, the sample was removed from the vial, washed with water and methanol (in that order), and dried under nitrogen.

Protein adsorption experiments were performed in a buffered solution of fibrinogen (1.0 mg/mL in PBS buffer pH 7.4). Samples were hydrated by soaking in PBS buffer for 30 min. This was followed by transfer into wells of a cell well plate containing protein solution. Sodium azide (0.2 mg/mL) was added to the protein solution as a bacteriostat. After 24 h of exposure time samples were removed from the solution, washed with PBS buffer and millipore water and dried under nitrogen.
5.2.2 Instrumental Analysis:

Ellipsometric thicknesses were measured on a LSE stokes ellipsometer (Gartner Scientific Corporation) operating with a 632.8 nm He-Ne laser at a 70° incident angle. Ellipsometric constants (Ns, Ks) were measured for each sample just after piranha cleaning. Refractive index of 1.46 was assumed for the organic film on Silicon surface and a three phase model was used to determine the thickness. Thicknesses were measured at four different spots per sample. The standard error of measurement is ± 2 Å.

Contact angle measurements were performed on a Ramé-Hart goniometer. Both, advancing and receding contact angles were measured for water drops (drop volume 4 μL) under ambient environment. Measurements were done at three different locations across each sample. The reliability in this measurement is ± 3°.

X-ray photoelectron spectroscopy data was obtained was on a Physical Electronics (PHI 5700) XPS/ESCA system. Samples were irradiated with monochromatic X-ray beam (1486.6 eV, Al source) inside a UHV chamber at 5 \times 10^{-9} Torr operating pressure. Data were collected at a photoelectron take-off angle of 45°. XPS photoelectron lines were referenced against C1s signal at 284.50 eV. Survey spectra were obtained using a 50 μm diameter beam (beam power 12.1 W) and pass energy of 112 eV. High resolution C1s scans were obtained using pass energy of 26 eV and acquisition time of 6 min (Beam size 100 μm; beam power 24.9 W). For measurement of protein adsorption, N1s scans were performed at pass energy of 55 eV and acquisition time 3 min (Beam size 100 μm; beam power 24.9 W). Measurements were performed at 2 different locations on the same sample; 2 samples per data point.
Data analysis was performed using PHI Multipak™ software provided by the instrument supplier. For curve fitting of C(1s) and N(1s) peak, full width at half maximum (FWHM) was kept constant for all components of a spectrum.
Chapter 6
Results Section

1) Kinetics of surface-initiated ATRP:

Fig. 6.1 shows the behavior of different monomers in terms of kinetics of polymerization (Monomer Concentration = 0.5 M irrespective of $n$; catalyst concentration same for all monomers). After initial short time period of transition, there is linear increase in the thickness of the polymer films. Rate of growth of polymer film decreases with increase in EG chain length ($n$), with the exception of $n = 9$, which has a greater rate than $n = 5$.

Fig. 6.2 shows the kinetics data normalized by converting thickness into more meaningful quantity – surface density of monomer units. ($\sigma$)

$$\sigma = \rho \cdot \tau / (MW)$$

where, $\rho =$ polymer density

$\tau =$ thickness of polymer film (excluding thickness of the SAM)

and $MW =$ molecular wt. of the monomer.

It is expected that polymer densities of the polymers do not differ much. Assuming a value of $\rho = 1.0 \text{ g/cm}^3$, $\sigma$ has been calculated for various monomers. A plot of $\sigma$ vs. time can be used to compare the reactivities of the monomers.

The order of reactivity is: $4 < 5 < 9 < 10 < 23$. 
Figure 6.1: Kinetics of surface-initiated ATRP of monomers with different chain lengths.

Figure 6.2: Normalized kinetics data showing the increase in the surface density of monomer units as a function of polymerization time.
2) **Contact angle measurement:** Contact angle of a sessile water droplet on the surface is a measure of surface hydrophilicity. Lower $\theta_a$ (or $\theta_r$) means more hydrophilic surface.

Fig. 6.3 shows the variation of contact angle with thickness of polymer film. The advancing and receding contact angles for the bare initiator surface are $90^\circ$ and $88^\circ$ respectively. As the polymer film thickness increases, the contact angle decreases and stabilizes rapidly to the values for thick films. ($48^\circ$ and $42^\circ$ respectively)

Table 6.1 lists water contact angles for 10 nm thick polymer brushes of different monomers. This thickness was chosen because contact angles were found to be independent of thickness for films thicker than 5 nm. In general, the contact angle decreases as the EG content of the films is increased.

![Water contact angle vs. thickness of grafted polymer film](image)

*Figure 6.3: Water contact angle vs. thickness of grafted polymer film showing the increased hydrophilicity of thicker films. The data shown above is for poly(OEGMA)-OMe, n=9 films. (The curved lines serve as a guide to the eye only. No attempt has been made to fit the data)*
Table 6.1: Water contact angle measurements on poly (OEGMA) films

<table>
<thead>
<tr>
<th>Monomer</th>
<th>( n )</th>
<th>Water Contact Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \theta(\text{adv.}) )</td>
</tr>
</tbody>
</table>
| \[
\begin{align*}
\text{H}_3\text{C} & \quad \text{C} & \quad \text{O} & \quad \text{O} & \quad \text{CH}_3 & \quad \text{CH}_2 & \quad \text{OMe} \\
\end{align*}
\] | 4 | 53° | 43° |
| \[
\begin{align*}
\text{H}_3\text{C} & \quad \text{C} & \quad \text{O} & \quad \text{CH}_2 & \quad \text{CH}_2 & \quad \text{OH} \\
\end{align*}
\] | 9 | 48° | 42° |
|        | 23 | 38° | 35° |
|        | 1 | 63° | 50° |
|        | 5 | 56° | 53° |
|        | 10 | 56° | 42° |

3) **Characterization of polymer films by XPS**: XPS was used for characterization of the polymer films. High resolution C (1s) scans were taken for polymer films thicker than 10 nm. (Spectra attached for reference; next page). The C 1s core-level spectrum of each Poly(OEGMA) film can be curve-fitted with three peak components (binding energies at about 284.6, 286.2, and 288.7 eV) corresponding to the C-H, C-O, and O-C=O species, respectively. As the EG chain length increases, there is an increase in intensity of the C-O peak at Binding energy of 286.2. However the ratios of peak areas are not in exact agreement with the stoichiometric ratio of functional groups because of the heterogeneity of the polymer films.

Figure 6.4 a and b present the XPS data for characterization of Poly (OEGMA-OMe) and poly(OEGMA-OH) films respectively.
Figure 6.4 a: XPS data for poly(OEGMA-OMe) films
Figure 6.4 b: XPS data for poly(OEGMA-OH) films
4) Protein adsorption measurements by XPS:

We have used XPS to monitor the adsorption of proteins on poly (OEGMA) films. The adsorption of protein on surface should manifest itself as an increase in the intensity of N (1s) peak in the XPS spectrum. The underlying substrate (i.e. Silicon surface + SAM + Polymer films) does not contain any nitrogen. Therefore, any increase in nitrogen content as seen by XPS is solely due to the adsorbed proteins. Figure 6.4 shows the XPS data for measurement of protein adsorption. The low levels of N (1s) signal for OEGMA films are an indication of their protein resistant properties.

![Graph showing N(1s) intensity for different samples](image)

Fig 6.4: a) Albumin adsorption on various control surfaces and poly(OEGMA) films as measured by the increase in N(1s) signal in XPS.
Fig 6.4: b) Lysozyme adsorption on various control surfaces and poly(OEGMA) films as measured by the increase in N(1s) signal in XPS.

Fig 6.4: c) Fibrinogen adsorption on various control surfaces and poly(OEGMA) films as measured by the increase in N(1s) signal in XPS.
5) Effect of thickness on wetting and protein resistant properties of the polymer film:

In order to observe the effect of thickness on wetting behavior of these films, I measured water contact angles for films of thickness 0 to 15 nm. Fig. 6.5 shows contact angle data for films prepared using the OEGMA 9 monomer. The water contact angles decreased rapidly in the range 0 to 2.5 nm (thickness excluding the SAM layer) and stabilizes to the value for thick (thickness > 10 nm) films. The films are heterogeneous in this thickness range (0-2.5 nm), probably due to lack of control over polymer growth during the initial stage of ATRP.

Next, to determine the required minimum thickness, protein adsorption on films with thickness ranging from 0 to 5.0 nm (excluding the initiator thickness) was observed by Ellipsometry (Figure 6.6). Films with thickness as small as 3 nm are found to be protein resistant.

![Water contact angle vs. thickness of poly(OEGMA-OMe) (n=9) film.](image)
Figure 6.6: Effect of polymer film thickness on protein resistant properties: increase in film thickness after exposure to fibrinogen solution. Polymer = Poly(OEGMA-OMe), n=9)
Summary and Conclusions:

Surface-initiated ATRP is applied for the synthesis of grafted poly (OEGMA) films. These films are hydrophilic and protein resistant due to the presence of EG chains. The effects of chain length and end group of the monomers on kinetics of polymerization and the protein resistance of the films are evaluated. The minimum thickness necessary for a poly (OEGMA) film to be protein resistant is also determined.

Bibliography

Appendix A

Instrumental Methods

1. **Ellipsometry[1]**

Ellipsometry is a very sensitive measurement technique for determination of (optical) thickness and/or the refractive index of thin films. An ellipsometer measures the change in polarization of an elliptically polarized light beam upon reflection.

![Figure 1: Optical model of a film for the determination of thickness by ellipsometry](image)

Fundamental equation of Ellipsometry is:

\[
\frac{R^P}{R^S} = \tan \psi \ e^{j\Delta} \ ..................(1)
\]

where, \(R_p\) (\(R_s\)) is the complex ratio of outgoing wave amplitude to the incoming wave amplitude for the components parallel (perpendicular) to the plane of incidence.

\[
\tan \psi = \left| \frac{R^P}{R^S} \right| \ ..................(2)
\]

\[
\Delta = \delta_1 - \delta_2 \ ..................(3)
\]
δ₁ (δ₂) is the phase difference between the parallel and perpendicular component of the incoming (outgoing) wave.

Using Fresnel’s equations, it is possible to express the left hand side of eqn (1) in terms of the refractive indices (\( \bar{N}_i = n_i - jk_i \ i = 1,2,3 \)), angle of incidence (\( \Phi_1 \)), wavelength (\( \lambda \)) and thickness of the film(d) (figure 1). Equating the real and imaginary parts, two independent equations are obtained which, can be solved for two unknowns.

Because ellipsometry measures the ratio of two values, it can be highly accurate and very reproducible. However, due to the periodic nature of eq 1, it can only be used up to certain thicknesses (i.e. period of measurement). Also, accuracy of the measurement depends on the assumed optical model.

2. **Contact angle measurements:**[2]

![Figure 2: Schematic illustration of the relationships between contact angle (\( \theta \)) and the three relevant interfacial energies (\( \gamma \))](image)

Contact angle is defined as the angle between the liquid-vapor interface and the liquid–solid interface at the solid-liquid-vapor three-phase contact line. It is a measure of the attraction of molecules within the droplet to each other versus the attraction or
repulsion those droplet molecules experience towards the surface molecules. In other words, contact angle analysis characterizes the wettability of a surface. It is one of the most sensitive and inexpensive surface analysis techniques.

The relationship between the surface free energies at equilibrium $\gamma_{LV}$, $\gamma_{SV}$, and $\gamma_{SL}$ is given by Young’s equation (eq 4).

$$\gamma_{LV} \cos \theta = \gamma_{SV} - \gamma_{SL} \quad ----- (4)$$

$\theta$ in Young’s equation is the equilibrium contact angle. However, in practice, dynamic contact angles are measured- the droplet is expanded by successive addition of liquid until a plateau in the contact angle is reached. This plateau is known as the “advancing contact angle”. Immediately following the advancing contact angle experiment, the process is reversed to obtain a ‘receding contact angle’ value. (Equal volumes of liquid are successively retracted from the drop.) The advancing and retreating angles are usually not equal; there is normally a hysteresis resulting from surface roughness, chemical heterogeneity, surface reorganization, etc.

3. X-ray photoelectron spectroscopy: [3]

X-ray photoelectron spectroscopy, known as XPS or ESCA, is an ultrahigh vacuum technique for determination of surface chemical composition. An XPS spectrum is obtained by irradiating the sample with ‘soft’ monochromatic X-rays (1486.6 eV for Al K\textsubscript{α} source) and analyzing the energies of photoelectrons ejected. The emitted electrons have kinetics energies given by:

$$KE = h \nu - BE - \varphi_s \quad ------------------------------- (5)$$
Because each element has a unique set of binding energies, it is possible to identify elements present near the surface region (except H and He). Small variations in BEs are observed due to differences in chemical potential and polarizability of compounds. Thus, chemical state of the element can be identified. The typical analysis depth of XPS is 10 nm, and its elemental sensitivity is 0.1 atomic %.

Figure 3: Schematic illustration of an X-ray photoelectron spectrometer.
Appendix B

Self-assembled monolayers

While numerous methods exist for modification of substrate properties by coating the substrate with bulk of other material, there is continuing interest in the development of coatings that are assembled at molecular level. Examples of these “nanostructured” assemblies are- Langmuir-Blodgett films, self-assembled mono layers and multilayers, and molecular beam epitaxy of evaporated organic molecules. An attractive feature of such coatings is that surface properties can be tailored by manipulating molecular structure of building blocks.

Self-assembled monolayer:[2]

Self-assembled monolayers (SAM) are formed by chemisorption of a surfactant like compound on a substrate. The surfactant like molecule has a head group that can form chemical bond(s) to the atoms on the surface of substrate (figure 4). SAMs are similar in structure to Langmuir-Blodgett monolayers but are more stable and uniform. SAMs are more readily prepared because the driving force for their formation is a spontaneous chemical reaction at the interface.
Figure 4  a) Molecular structure of a surfactant like molecule capable of forming a self-assembled monolayer on a surface. Head group can form chemical bond(s) with the surface; tail group determines the surface properties.

b) Thickness of a SAM is determined by the length of tether (typically a hydrocarbon chain). SAM structure is also stabilized by the van der Waal interactions between neighboring molecules.

In this project, SAMs are used to prepare a surface with uniformly distributed bromine atoms. To achieve this goal, a long chain molecule 1 with trichlorosilane at one and bromine at the other end is synthesized. SAMs of uniform composition are obtained by immersing a piranha cleaned Silicon wafer in millimolar solution of 1 for 20 hours.
(11-(2-Bromo-2-ethyl)propionyloxy)undecyltrichlorosilane [1]

Figure 5:
a) Trichlorosilane coupled initiator for self-assembly on Si surface.
b) Self-assembly of 1 on silicon surface from 4mM solution in anhydrous toluene.

Reactions conditions play an important role in obtaining high-quality SAMs. Presence of water in the SAM solution is undesirable because, water will cause bulk polymerization by hydrolysis of trichlorosilane group. This can be avoided by using anhydrous solvents and performing the reaction in a drybox. Water is however necessary in form of thin film adsorbed on hydrophilic silicon oxide. Thus, degree of hydration of the silicon will significantly affect the thickness of SAMs obtained.
Figure 6: Effect of the presence of bulk water on the quality of SAM

Thickness of self-assembled monolayer of the initiator (compound 1) is 20.4 Å and the advancing and receding contact angles for a water droplet on these SAMs are 90° and 88°, respectively.

References: